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ON THE RELATION OF THE REDUCING POWER OF NORMAL URINE TO THE AMOUNT OF CERTAIN NITROGEN COMPOUNDS PRESENT.

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THE reducing power of normal urine is easily observed by the application of certain reagents and was at one time supposed to be largely or mainly due to the presence of dextrose or some similar body. In fact, based on the reducing power alone, estimates of the amount of carbohydrates in urine were frequently made and are still occasionally found in the text-books of urine analysis and physiological chemistry. Later, doubt was thrown on this conclusion and the presence of even traces of sugar in normal urine was disputed. Seegen[1] made many experiments in this direction and came to the conclusion that if any sugar at all is present it cannot be in amount above 0.006 per cent. The same result essentially was reached by several others, but in most cases the methods of examination employed were open to criticism as they did not provide for the actual separation and identification of a sugar, supposing it present.

Meanwhile E. Fischer[2] proposed the reaction for the separation of sugars by the phenylhydrazine test, and von Jaksch[3] and

1 *Ztschr. physiol. Chem.*, **9**, 332.
2 *Ber. d. chem. Ges.*, **17**, 579 and **20**, 82.
3 *Ztschr. anal. Chem.*, **24**, 478.

others applied it to the identification of sugar in urine. Baumann[1] showed the ready production of benzoic esters of dextrose and this general method was soon applied in urine examination by Wedenski,[2] Roos,[3] Salkowski,[4] and others. The amount of the pentabenzoic ester found in this way was quite variable but always small. In Salkowski's test the range was from 1.22 grams to 3.36 grams in 1000 cc. of urine representing the whole day's excretion. In Wedenski's experiments the maximum amount was ten times the minimum found. Later Baisch made two important contributions on the subject of the nature and amount of the carbohydrates in the urine[5] and places the content of these bodies somewhat higher than Seegen, but still very low. His results give the average excretion of reducing carbohydrates as 0.100 gram to the liter. About the same time Allen published an interesting paper on the subject[6] and reached nearly the same result.

It is quite evident from the foregoing that while the existence of sugar in the urine may be looked upon as settled, the amount is very small and far from accounting for the total reduction. This has been reported by some authors as corresponding to 0.15 per cent. of dextrose while others place it as high as 0.4 per cent., in the mean.

NATURE OF THE REDUCING BODIES.

When speaking of reduction the behavior toward some metallic solution, generally toward copper oxide, is usually in mind. Among substances, other than sugar, which occur normally in urine the most important from the standpoint of this behavior are doubtless uric acid and creatinin. Glycuronic acid is often referred to as having an important action here, but the amount ordinarily present is too small to be practically considered in comparison with the others. The reducing power of uric acid has been long known and under certain definite conditions an equation may be written expressing the amount of oxygen absorbed in passing into several related bodies. The reducing

1 *Ber. d. chem. Ges.*, 19, 3218.
2 *Ztschr. physiol. Chem.*, 13, 122.
3 *Ibid.*, 15, 513.
4 *Ibid.*, 17, 229.
5 *Ibid.*, 18, 193 and 19, 339.
6 *Analyst*, 19, 178.

power of creatinin is fully as important as is that of uric acid, and besides it is more readily followed and measured.

It occurred to me, therefore, that some light could be thrown on the question of what part a sugar plays in the total reduction by determining as accurately as possible in a large number of normal urines the amount of uric acid and creatinin present, and calculating then the reducing power of these from their relations to the oxidizing solution previously determined. The difference between the total reduction and that due to these bodies would roughly measure the reducing power of the sugar present, provided no other substance has been overlooked which exhibits the same action. The possibility of the existence of such a substance or substances in the urine must, of course, be conceded, especially since the ratio of carbon to nitrogen present, as found by direct analysis, is much higher than that calculated from the sum of the determinable constituents present. The new substance recently separated from urine and called oxyproteic acid by Bondzynski and Gottlieb[1] is supposed by Pregl[2] to account for this ratio, and to be, after urea, the most important body in the urine. The analyses made by Pregl show it to contain about 30 per cent. of carbon and 8 per cent. of nitrogen, and to amount to 6 or 8 grams daily. He states, however, that the product, as separated by his methods of precipitation and purification, is quite devoid of any reducing action on alkaline copper solutions. Toepfer has also recognized this peculiar urinary acid[3] but claims that the amount found by Bondzynski and Gottlieb is too high, because the barium salt separated is always impure from presence of much coprecipitated matter. It is certainly remarkable that an acid as abundant as this is supposed to be, should have so long escaped detection, and this fact suggests caution in generalizing too much from the results of urine analyses, however carefully they may be made; but up to the present time no substances have been found in the urine in sufficient quantity to account for the reducing power save those mentioned, and it is from this, as a working basis, that the following investigation was undertaken.

While many substances have been employed as reagents in

1 *Centralblatt für Physiologie*, 11, 648.
2 Pflüger's *Arch.*, 75, 87, 1899.
3 *Centralblatt für Physiologie*, 11, 850.

this reduction test some form of copper solution has usually been found practically the most useful. A weak solution of methylene blue is reduced readily by sugar and creatinin in alkaline solution, and very slowly by uric acid, but as the reduced product is oxidized with extreme readiness in contact with air, with return of the blue color, the reagent is not suitable for quantitative measurements. A weak solution of safranine is also reduced by sugar in alkaline medium and apparently not at all by uric acid or creatinin, but the amount of sugar necessary to produce an appreciable effect is greater than that usually found in normal urine, so that this reagent is not practically available. Measurement of reduction by aid of bismuth or mercury salts is also possible, but, as is well known, the methods are lacking in delicacy.

Among the various copper solutions used in sugar analysis there are several which may be employed with very weak solutions corresponding to normal urine. The best of these appears to be some form of the ammonia-copper solution recommended by Dr. Pavy. This was first made by diluting 120 cc. of Fehling's solution with 300 cc. of strong ammonia and water enough to make 1 liter. Pavy assumed that each cubic centimeter of this solution oxidizes 0.5 mg. of dextrose, and has, therefore, one-tenth the strength of the ordinary Fehling solution, which is approximately true. Several modifications of the solution have been proposed with variations in the amounts of copper sulphate, ammonia, and fixed alkali. Finally, the Loewe solution containing glycerol has been made the basis of the dilution, instead of the Fehling solution containing a tartrate; the Purdy solution is made in this way.

As the oxidizing power of the solution changes with these variations, it is important to recognize the extent of this alteration and allow for it if necessary. The composition of several of the best known of the modifications is here given, the original Pavy solution being added for comparison. The volume is one liter in each case.

	Copper sulphate. Grams.	Sodium hydroxide. Grams.	Rochelle salt. Grams.	Glycerol. cc.	Ammonia. 0.90. cc.
Pavy	4.158	(7.20)	20.76	..	370
Hehner	4.502	15.6—19.5	22.49	..	370
Purdy	4.752	23.5 KOH(= 16.8 NaOH)	....	38	350
Peska	6.927	10.00	34.50	..	135

Variations in the oxidizing power of each solution follow mainly from alterations in the amount of fixed alkali or ammonia used, and this is shown in some tests to be now given. I made solutions as follows:

A.

Copper sulphate, cryst	5 grams.
Caustic soda (100 per cent.)	18 "
Glycerol	35 cc.
Water, to make	250 "

B.

Ammonia water	0.90 sp. gr.

These solutions were mixed in the proportions given below and used in the titration of a 0.2 per cent. dextrose solution in the usual manner. The results are shown in the following table:

TABLE I.—REDUCTION WITH AMMONIA VARIABLE.

Vol. of Sol. A. cc.	Vol. of Sol. B. cc.	Vol. of water. cc.	Sugar solution required. cc.	Mg. of $CuSO_4.5H_2O$ to 1 mg of $C_6H_{12}O_6$.	Mols. of CuO to 1 mol. $C_6H_{12}O_6$.
25	75	00	28.5	8.772	6.32
25	60	15	29.5	8.474	6.11
25	50	25	30.3	8.251	5.95
25	40	35	30.8	8.117	5.85
25	30	45	31.4	7.962	5.74

The oxidizing power varies greatly with the amount of ammonia present and is decreased with increase in the latter. The change amounts to about 10 per cent. of the whole in the limits given.

The effect of adding an excess of sodium or potassium hydroxide is even more marked as was found by experiments given below. The following solutions were made:

C.

Copper sulphate, cryst	5 grams.
Glycerol	35 cc.
Water, to make	250 "

D.

Strong ammonia water	0.90 sp. gr.

E.

Sodium hydroxide, 100 per cent	50 grams.
Water, to make	250 cc.

These solutions were mixed in different proportions and were used to test a dextrose solution of about 0.2 per cent. strength. The results obtained were as follows:

TABLE II.—REDUCTION WITH FIXED ALKALI VARIABLE.

Vol. of Sol. C. cc.	Vol. of Sol. D. cc.	Vol. of Sol. E. cc.	Vol. of water. cc.	NaOH in grams per liter. Grams.	Sugar solution required. cc.	Mols. of CuO to 1 mol. $C_6H_{12}O_6$.
25	35	25	15	50	34.8	5.18
25	35	20	20	40	33.3	5.41
25	35	15	25	30	32.0	5.63
25	35	10	30	20	30.4	5.93
25	35	5	35	10	28.7	6.28
25	35	2.5	37.5	5	28.0	6.44

We have here an extreme variation of about 25 per cent. in the oxidizing value of the copper solution. As the fixed alkali itself possesses marked oxidizing power, as shown, in fact, in our ordinary Moore's test for sugar in urine, the increase in alkali must add to the oxidizing power of the finished solution. Hehner has shown that the solution can be made and will oxidize perfectly without the addition of any fixed alkali, but the action is then very slow and the oxidation ratio still lower.[1]

These experiments indicate that to secure anything like uniformity in the results obtained by the Pavy solution or its modifications, care must be taken to employ definite and constant amounts of fixed alkali and ammonia. The statement of Allen ("Chemistry of Urine," p. 67, 1895) that considerable variation in the amount of caustic alkali and ammonia may be made in the Pavy solution without altering appreciably its oxidizing value, does not appear to be correct. Of the two cheap fixed alkali hydroxides, caustic soda is preferable to caustic potash and is much more commonly used.

Variations in the amount of glycerol or tartrate are of less importance, but still have an appreciable influence, as I have found in several trials. As the great excess of ammonia present is sufficient to hold the copper hydroxide in solution, it is not necessary to use a large amount of either.

In the Pavy and Purdy liquids the oxidizing power is apparently assumed to be independent of the strength of the sugar solution added. Pavy and Purdy assume the factor 8.316 grams of

[1] *Analyst*, 6, 219.

$CuSO_4.5H_2O$ to 1 gram of $C_6H_{12}O_6$. But Peska has shown that the factor varies over 2 per cent. with solutions ranging from 0.1 to 1 per cent. in strength.[1]

As in practical work it is desirable to employ a solution, 1 cc. of which oxidizes some simple unit amount of sugar. I have used one in the work below with a value of 1 cc. for each milligram of sugar oxidized in 0.2 per cent. solutions. It is made with the following amounts per liter:

Copper sulphate, cryst	8.166 grams.
Sodium hydroxide (100 per cent.)	15.000 "
Glycerol	25.000 cc.
Ammonia water (sp. gr. 0.9)	350.000 "
Water, to make	1,000.000 "

The value of this solution in copper oxide, CuO, is 2.6042 grams per liter, and 1 molecule of sugar = 5.88 molecules of CuO, as it is employed.

Of the solution, I use 50 cc. and dilute with water to 100 cc. To prevent too rapid an escape of ammonia and avoid reoxidation to some extent, I add to the mixture, while warming, enough pure white solid paraffin to make a layer of 3 or 4 mm. in thickness when melted. The burette tip for discharging the sugar solution or urine is made long enough to pass down the neck of the flask and below this paraffin. By boiling gently and adding the weak saccharine liquid slowly, very close and constant results may be obtained. At the end of the titration the paraffin is solidified by inclining the flask and immersing it in cold water, or by flowing cold water over it. The reduced liquid is then poured out and the cake of paraffin is thoroughly washed for the next test. A flask so prepared may be used for a hundred titrations. The solid paraffin is much preferable to the oil recommended by Allen and Peska. To prevent bumping and facilitate easy and uniform boiling, I add a few very small fragments of pumice-stone.

A solution made as above is not too strong in copper for accurate work, but the volume of ammonia necessary to hold a much larger amount of the reduced oxide in solution would render the process very inconvenient. The sugar employed in fixing the value of the above standard solutions was a very fine sample of

[1] *Ztschr. anal. Chem.*, **35**, 94.

pure crystal dextrose made for me by Dr. Gudemann, of the Chicago Sugar Refining Co. It was further purified by crystallizing from hot alcohol. After careful drying at 80° C., it was examined by a very accurate polarimeter and found to have a degree of purity not less than 99.9 per cent.

An ammoniacal solution made in this way and used with the layer of paraffin is preferable to the usual Fehling liquid in the titration of weak dextrose solutions. It cannot be conveniently used for strong solutions, however, because of the large volume of standard required to oxidize a small volume of the saccharine liquid.

The behavior of this solution with the weak sugar solutions being established, it remains to show how it acts with creatinin and uric acid.

CREATININ AND COPPER SOLUTIONS.

The importance of creatinin as a reducing body is commonly overlooked although referred to in Neubauer and Vogel's "Urine Analysis," and in other large works. This is partly due to the fact that the amount present is generally underestimated, as shown by the figures given in several of our best known handbooks of urine analysis.[1] The reducing effect of creatinin on alkaline copper solutions has been observed by Worm Müller,[2] Johnson,[3] and others. Müller places the reducing power low, 1 molecule of creatinin to not over 0.75 molecule of copper oxide.

Johnson states that 4 molecules of ordinary creatinin have the same reducing action on copper salts as 2 molecules of grape-sugar, and that this reduction plus that due to the uric acid will account for the whole of the reducing action found in normal urine. The presence of sugar is disputed. The results of Müller and Johnson, are widely divergent, which is doubtless due to essential differences in the methods of observation. The reducing power of creatinin is shown only after long warming in the ordinary Fehling titration, and at the outset the cuprous oxide formed is held in solution, which is a disturbing element in making the test. But with the weak ammoniacal solution

[1] See on this point, Allen, *loc. cit.*
[2] *Ztschr. anal. Chem.*, **21**, 610.
[3] *Chem. News*, **55**, 304.

used for the sugar the behavior is quite different. The reduction appears to proceed as regularly and normally as with sugar itself. I have made a number of tests with solutions of different strengths, but all weak, and find that about 92 mg. of creatinin are required for 50 cc. of the standard copper solution containing 2.604 grams of CuO to the liter. This corresponds almost exactly to the proportion, 1 molecule $C_4H_7N_3O$: 2 molecules CuO, which is about one-third the reducing power of dextrose under the same conditions. The result is much larger than that of Worm Müller, but lower than the figures given by Johnson. The actual reducing values are given in the table below. The creatinin used was prepared in the laboratory and was practically pure.

TABLE III.—REDUCING POWER OF CREATININ.

Creatinin in 100 cc. Mg.	Copper solution taken and diluted to 100 cc. cc.	CuO equivalent. Mg.	Creatinin solution used. cc.	Creatinin to 130.2 mg CuO. Mg.	Mols. CuO to 1 mol. $C_4H_7N_3O$.
50	25	65.1	92.5	92.5	1.998
50	25	65.1	94.0	94.0	1.967
120	50	130.2	76.0	91.2	2.026
120	50	130.2	77.0	92.4	2.000
				Mean,	1.998

The oxidation of creatinin by alkaline copper solutions is usually explained on the assumption that oxalic acid and methyl guanidin are formed, the creatinin being first converted into creatin by the alkaline solution,

$$HN{=}C{=}NH_2.NCH_3.CH_2CO_2H + O_2 = C_2H_2O_4 + HN{=}C{=}NH_2.NHCH_3.$$

In the above experiments, however, the amount of oxygen absorbed corresponds with only half that necessary for this equation, and under the condition none can come from the air. The reaction must therefore take place in a different manner, which appears all the more probable in view of recent work by E. Wörner,[1] and Toppelius and Pommerehne,[2] who found that the reduction is variable with time of boiling with strong Fehling solution. With weak solutions the reduction is slow, but on boiling 5 cc. of a weak creatinin solution an hour with 60 cc. of strong Feh-

[1] *Ztschr. physiol. Chem.*, **27**, 1.

[2] *Archiv. der Pharmacie*, **234**, 380.

ling solution a much greater effect is observed. Under such conditions 1 molecule of creatinin appears to reduce over 4 molecules of CuO. It is quite possible that the oxidation of creatinin is preceded in weak solution by hydrolysis with formation of ammonia and methyl hydantoin and that this body is afterwards oxidized in several stages. The reduction of 4 molecules of CuO would correspond to the oxidation of the acetic acid group in creatin to oxalic acid, as required by the equation written above, but in the ordinary application of the copper tests in urine analysis no such degree of oxidation is likely.

URIC ACID AND COPPER SOLUTIONS.

The behavior of uric acid with alkaline copper solutions has been described by several chemists and notably by Riegler[1] who studied the reaction with Fehling solution. He found, as a mean result of a number of experiments, that 1 gram of uric acid yields cuprous oxide corresponding to 0.800 gram of copper. On the assumption that 1 molecule of uric acid reduces 2 molecules of copper oxide the reduced copper obtained should amount to 0.7556 gram; the reduction therefore goes a little further than this theoretical relation.

The reducing action seems to be much more readily followed in the ammoniacal solution, however, and several tests were made to establish the relation under such conditions. The following are the details in tabular form:

TABLE IV.—REDUCING POWER OF URIC ACID.

Uric acid in 100 cc. Mg.	Copper oxide taken.	Uric acid solution used. cc.	Uric acid to 130.2 mg CuO. Mg.	Mols. CuO to 1 mol. $C_5H_4N_4O_3$.
80	39.8	36	94.2	2.92
80	65.1	58	92.8	2.96
120	130.2	76.5	91.8	2.99
120	65.1	37.8	90.8	3.03
			Mean,	2.98

The mean result of 1 molecule of uric acid to 2.98 molecules of copper oxide is essentially in the relation of 1 : 3, or 1 molecule of the acid to 1.5 atoms of oxygen absorbed. This is a larger amount of oxygen than corresponds to the reactions usually given for the oxidation of uric acid which is generally

[1] *Ztschr. anal. Chem.*, 35, 31.

represented as taking place primarily in one of two directions, leading either to allantoin or to alloxan and urea. These reactions require each one atom of oxygen as follows :

$$C_5H_4N_4O_3 + O + H_2O = CO_2 + C_4H_6N_4O_3$$
$$C_5H_4N_4O_3 + O + H_2O = C_4H_2N_2O_4 + CON_2H_4.$$

Secondary reactions, however, doubtless take place resulting in the further oxidation of one or the other of these products and using more oxygen. Thus, from alloxan parabanic acid and other substances may be derived, and possibly are derived in the oxidation in question. A reaction leading to parabanic acid from the partial oxidation of the alloxan in the above case may be represented in this way :

$$2C_5H_4N_4O_3 + 3O + 2H_2O =$$
$$2CON_2H_4 + C_4H_2N_2O_4 + C_3H_2N_2O_3 + CO_2.$$

Whether this reaction represents the course of the oxidation or not it remains true that the uric acid takes up more oxygen than is required by the simpler reactions given above. This is true also of a reaction in sulphuric acid solution referred to below.

Having described the three important substances in urine which exert a reducing action on the ammoniacal copper solution, it remains to explain how the last two mentioned, along with urea and ammonia, the other important products of nitrogenous excretion, were practically determined in the investigation in hand. The amounts of these last substances are of interest in connection with the actual reducing compounds.

DETERMINATION OF CREATININ.

This body is most accurately obtained by precipitation from the prepared urine in the form of zinc chloride double salt. This is the method of Neubauer modified by Salkowski, but it is desirable to use as large a volume of urine as possible. In the work below I used, when available, 480 cc. which was treated with barium hydroxide to faint alkaline reaction and precipitated with barium nitrate in the cold. In a few cases milk of lime and calcium chloride were used in this preliminary treatment. The volume was then made up to 600 cc., filtered after half an hour, and of the filtrate 500 cc., representing 400 cc. of the original urine, was taken for the further work. Care was observed to

secure and maintain a nearly neutral reaction in this liquid so as to avoid, in the following evaporation, the conversion of creatinin into creatin. The final precipitate of the zinc chloride salt was collected on a Gooch crucible, dried at 100°, and weighed. From this weight that of the pure creatinin was calculated and this is given in the table below.

DETERMINATION OF URIC ACID.

Of all the methods now available, the Fokker-Hopkins process seems to give the most regular and trustworthy results. 100 cc. of urine are precipitated with about 30 grams of pure ammonium chloride, or enough to produce a saturated solution. The beaker containing the precipitate is allowed to stand in a cool place over night. Then the precipitate is collected on a filter and washed thoroughly with a saturated solution of ammonium sulphate to remove all chlorine. The filter is perforated and by aid of a jet of hot water the precipitate is washed into a flask. About 100 cc. of the water should be used. To this turbid liquid 20 cc. of pure strong sulphuric acid is added, and then, without delay, twentieth-normal permanganate solution from a burette until a faint pink tinge is secured which is permanent several seconds. For this stage of the reaction Hopkins gives 1 cc. of the twentieth-normal permanganate as corresponding to 3.75 mg. of uric acid. By waiting and adding more permanganate from time to time, as the color fades, a considerably larger volume may be used, for which no factor has been worked out. For the direct oxidation, using 1 atom of oxygen to 1 molecule of uric acid, each cubic centimeter of the twentieth-normal permanganate solution should correspond to 4.2 mg. of the acid instead of 3.75 mg. The results obtained by taking a later reading correspond more nearly to an oxidation with 1.5 atoms of oxygen to each molecule of acid, but they are not definite enough for calculation. The Hopkins factor has therefore been used, and, it is believed, with a considerable degree of accuracy, since numerous blank experiments were carried out with pure uric acid to fix the point for observation of the end color.

DETERMINATION OF UREA.

This has been carried out by the two common methods, the Liebig process by titration with mercuric nitrate, giving properly

a rough measure of the total nitrogen rather than the urea itself, and by the Knop-Hüfner hypobromite or hypochlorite process. The results of the first method are always too high if calculated as urea, while those of the latter are too low. As the last process was carried out it was assumed that 95 per cent. of the urea present was decomposed normally according to the equation

$$CON_2H_4 + 3O = CO_2 + N_2 + 2H_2O,$$

the calculations being made accordingly from the experimental results. The observations were made in a Lunge nitrometer, the gas volumes obtained being always reduced to standard conditions. Even with the various corrections the results were considerably lower than those by the Liebig process because of the inherent positive errors in the latter. Something will be said about this below when the experimental results are discussed.

DETERMINATION OF AMMONIA.

Of all the processes recommended for the determination of the small amounts of ammonia found in urine the most reliable is the old Schloesing method of liberation by milk of lime and absorption by standard acid, under a bell-jar. The urine, about 50 cc., is measured into a glass crystallizing dish and mixed with an excess of milk of lime. Over this dish a glass evaporating dish containing a small known volume of standard sulphuric acid is supported on a triangle. The whole is covered without delay with a clean glass bell-jar, resting on a glass plate and is allowed to stand three days for the liberation of the ammonia, and its absorption by the titrated acid. At the end of the time the bell-jar is rinsed out with a little water which is added then to the dish with the standard acid. This is finally titrated and the loss in acidity measures the ammonia absorbed; while the process is very slow little or no risk is incurred of decomposing urea or other substance containing nitrogen and thus liberating an excess of ammonia. It is, of course, necessary to operate on fresh urine, as the object is to measure that normally present, and not that which may be made by the alkaline fermentation of urea. The results are always a little low, because a small portion of the ammonia escapes either liberation or absorption. To determine the probable error here a number of experiments were made with weak ammonium chloride solutions, corresponding in ammoniacal

strength to normal urine. These experiments gave very concordant results from which it appeared that after three days' time, at the temperature of the tests, 90 per cent. of the ammonia present was liberated and absorbed by the acid. In all the experiments below the results obtained were corrected by the aid of this factor.

Table V, following, gives the result of examinations of a number of normal urines, embracing determinations of the above-described constituents. These urines were collected so as to secure the whole day's excretion and were kept cold until the full twenty-four hour sample was obtained. The analysis was then begun immediately, and finished as speedily as possible. Fifty cc. of the standard copper solution were always employed in the reduction test, and the following table gives the volume of urine required to reduce this under the conditions described above. The reaction of the mixed day's urine was always acid and all samples, except No. 7, were of the usual normal yellow color. This sample was dark, probably because of its concentrated condition. It will be noted that the average total volume is smaller than is usually assumed as the mean daily excretion.

TABLE V.—RESULTS OF URINE ANALYSES.

No. of sample.	Excretion in twenty-four hours.	Specific gravity at 20°.	Urine required to reduce 50 cc. of copper solution.	Ammonia from 1000 cc. of urine.	Uric acid from 1000 cc. of urine.	Creatinin from 1000 cc. of urine.	Urea from 1000 cc. of urine by the Liebig process.	Urea from 1000 cc. of urine by the Knop-Hüfner process.
			cc.	Mg.	Mg.	Mg.	Grams.	Grams.
1	1230	1.031	21.0	645.0	840.0	1930	30.50	....
2	1450	1.035	21.2	522.0	743.0	1876	25.60	....
3	845	1.027	23.6	654.8	832.5	1268	38.80	....
4	1920	1.019	22.5	427.0	646.0	851	25.75	....
5	1950	1.023	20.2	465.0	678.0	1681	24.74	23.72
6	1200	1.025	20.5	593.1	619.0	1195	29.69	27.66
7	765	1.024	18.9	526.6	637.5	961	31.10	27.46
8	1775	1.020	58.0	227.7	....	653	12.90	....
9	1020	1.030	14.9	858.8	971.2	1750	39.18	37.27
10	1280	1.018	27.3	408.0	423.7	1016	17.57	14.83
11	1090	1.024	23.3	631.0	510.0	1374	25.04	24.54
12	1190	1.025	25.6	431.7	611.0	1381	32.72	29.72
13	915	1.025	21.1	711.7	630.0	1604	28.17	25.56

No. of sample.	Excretion in twenty-four hours.	Specific gravity at 20°.	Urine required to reduce 50 cc. of copper solution.	Ammonia from 1000 cc. of urine.	Uric acid from 1000 cc. of urine.	Creatinin from 1000 cc. of urine.	Urea from 1000 cc. of urine by the Liebig process.	Urea from 1000 cc. of urine by the Knop-Hüfner process.
			cc.	Mg.	Mg.	Mg.	Grams.	Grams.
14	1440	1.015	27.8	522.0	491.0	868	17.67	15.85
15	1220	1.029	21.2	716.0	671.0	757	26.46	20.94
16	1200	1.025	19.7	711.6	712.5	1735	32.11	31.23
17	800	1.030	17.9	669.0	693.8	1681	34.23	30.29
18	900	1.022	23.7	488.7	581.3	1239	21.91	17.83
19	1100	1.027	18.1	507.7	581.2	1898	26.32	21.73
20	835	1.032	17.1	1001.0	1020.0	1417	39.10	34.68
21	900	1.023	17.4	569.4	626.0	2224	26.63	23.66
22	970	1.026	23.4	374.8	521.0	976	18.61	16.15
23	1600	1.018	28.0	601.6	416.2	926	19.30	17.60
24	1120	1.027	20.7	811.3	701.2	1477	24.55	22.47
Means,	1167	1.025	23.0	602.0[1]	658.7	1392[1]	27.68[1]	24.37

The analytical results as obtained are given in Table V. These, as might be expected, are quite variable, and at first glance do not reveal any relationship that appears characteristic. For better comparison I have stated the amounts of urea, uric acid, ammonia, and creatinin found in grams or milligrams per 1000 cc. rather than for the excretion of twenty-four hours. In Table VI, I have calculated the total reducing power in terms of CuO for 1000 cc. of each urine, and have then done the same for the uric acid and creatinin, basing the calculation on the assumption that under the conditions of the experiments each molecule of uric acid reduces 3 molecules of copper oxide and each molecule of creatinin reduces 2 molecules of the oxide. The sum of these two reductions is given in a separate column, and finally the ratio of this to the total reduction is given in the last column.

[1] No. 8 not included.

TABLE VI.—RELATION OF REDUCING POWERS.

No. of sample.	Total reducing power of 1000 cc. of urine in grams of CuO.	Reducing power of the uric acid in 1000 cc. of urine in grams of CuO.	Reducing power of the creatinin in 1000 cc. of urine in grams of CuO.	Sum of the uric acid and creatinin reductions.	Relation of the uric acid and creatinin reductions to total reduction.
1	6.200	1.194	2.719	3.913	0.631
2	6.141	1.056	2.643	3.699	0.602
3	5.517	1.184	1.786	2.970	0.538
4	5.787	0.911	1.199	2.110	0.365
5	6.446	0.964	2.368	3.332	0.517
6	6.351	0.880	1.684	2.564	0.404
7	6.889	0.906	1.354	2.260	0.328
8	2.245	....	0.920	....	....
9	8.738	1.381	2.370	3.751	0.429
10	4.769	0.602	1.432	2.034	0.427
11	5.588	0.725	1.936	2.661	0.476
12	5.086	0.869	1.946	2.815	0.554
13	6.171	0.896	2.260	3.156	0.511
14	4.684	0.698	1.223	1.921	0.410
15	6.141	0.954	1.066	2.020	0.329
16	6.689	1.013	2.444	3.457	0.523
17	7.291	0.986	2.369	3.355	0.460
18	5.494	0.826	1.746	2.572	0.468
19	7.192	0.825	2.674	3.499	0.487
20	7.614	1.417	1.997	3.414	0.448
21	7.483	0.890	3.133	4.023	0.538
22	5.564	0.741	1.375	2.116	0.380
23	4.650	0.592	1.305	1.897	0.408
24	6.290	0.997	2.081	3.078	0.489
Means,	6.204	0.935[1]	1.961[1]	2.896	0.466

From the mean values given at the foot of the last table several interesting relations may be established. As in the ammoniacal solution 1 molecule of dextrose reduces 5.88 molecules of copper oxide, the above average reduction corresponds to 2.836 grams of sugar per liter, or about 0.28 per cent. The uric acid amounts to 0.658 gram per liter, or 0.065 per cent. The creatinin is equivalent to 1.392 grams per liter or 0.136 per cent. The data of the last column show that a large portion of the total reduction is due to the action of these latter products.

[1] No. 8 not in averages.

This, in the mean, amounts to 46.6 per cent., which calculated as sugar is equivalent to 1.322 grams per liter, leaving 1.514 grams as the amount of sugar possibly present. This, however, represents a maximum value, as there are unquestionably traces of other bodies present which, like the uric acid and creatinin, exert a reducing action. If the effect of these could be estimated it is likely that the reducing power, in the mean, would be found to be pretty evenly distributed between the saccharine and non-saccharine products.

It is not possible to draw any very exact general conclusions from the figures of the above table connecting the numerical values with the character of the food of the individual or with any other factor. Most of the urines were obtained from young men, students or teachers, and all pretty well nourished. It may be noticed, however, that the urines showing the highest reduction ratio for uric acid, and creatinin, as compared with the total reduction, were from men with the strongest physique with a diet containing much meat. On the other hand the lowest uric acid, and creatinin reductions correspond to cases of slighter physique and lower nutrition. Urines Nos. 1 and 2 were from a man consuming a diet largely of meat. No. 15 was from the same individual some weeks later after a change of diet to bread and vegetables largely. But from most of the urines no characteristic relation is apparent; another series of investigations is in progress in which the question of food in relation to the reducing power is being more closely determined.

RATIO OF UREA TO URIC ACID.

This ratio is more important and more characteristic than is the absolute amount of the acid. In the older works on urine analysis or physiological chemistry it was always stated too high, and usually as 50 : 1 or 60 : 1. This is due to the fact that the amount of uric acid was generally underestimated, while that of urea was naturally overestimated if found by the Liebig process, and not corrected.

In making an examination of a fraction of the day's excretion, instead of on the mixed twenty-four hour sample, a false ratio is also usually found because the urea and uric acid are not eliminated at a constant rate following ingestion of food. The ratio

from the above table is 36.9 : 1 if we consider the urea as measured by the Knop-Hüfner process and 42 : 1 if we take the uncorrected values for urea as found by the Liebig process. The results here are in the mean about 10 per cent. high as may be shown when we take into consideration the effect of the other nitrogenous bodies, especially the ammonia, uric acid, and creatinin, on the mercuric nitrate solution. With such a correction subtracted the amount of urea approaches closely that found by the gas volume method and the ratio becomes again about 37:1; this result is in accord with the average normal ratio as found in experiments on the excretion of the twenty-four hours as made by Hopkins and others. See an interesting paper by Hopkins and Pope[1] in which variations in the excretion of urea and uric acid in relation to kind of food and time of meals is discussed.

THE AMOUNT OF AMMONIA.

The ammonia in 1000 cc. of urine, as shown by the above figures, is, in the mean, 602 mg., but the individual variations are between 228 and 1001 mg. These correspond roughly to the variations in the other nitrogen bodies. The average daily excretion is seen to be 703 mg. The daily extremes are 364 and 908 mg. The average correction to be applied to the Liebig urea titration on account of the ammonia present is about 1.2 cc. of the usual standard mercuric nitrate solution. This result was found in experiments carried out on the titration of urea solutions containing small amounts of added ammonia, and is somewhat lower than the factor given by Feder.[2] Experiments are now in progress to determine more definitely the behavior of uric acid and creatinin in this titration, the statement in Neubauer and Vogel's Harnanalyse[3] requiring, apparently, a slight correction.

THE DAILY AVERAGES.

The numbers above given are expressed, for convenience in comparison, in grams or milligrams per liter. The values for the daily excretion are often interesting and these will now be given as calculated from the volumes collected in the twenty-

[1] *Journal of Physiology*, **23**, 271.
[2] Neubauer and Vogel's Harnanalyse, 9th German edition, p. 519.
[3] *Loc. cit.*

four hours as shown by the figures of the second column in Table V.

TABLE VII.—MEAN RESULTS FOR DAILY EXCRETION.

Volume excreted	1167.00	cc.
Total reduction, equivalent to	3.31	grams $C_6H_{12}O_6$.
Uric acid and creatinin reduction equivalent to	1.54	" "
Remaining reduction equivalent to	1.77	" "
Amount of ammonia	0.703	gram.
Amount of urea (mean of gas method and Liebig, corr.)	29.75	grams.
Amount of creatinin	1.624	"
Amount of uric acid	0.799	gram.

The urines examined in the above experiments were all normal and represent the excretion from average meat and vegetable diet. In a following paper I will give results obtained from urines of consistent vegetarians, noting the same relations. Most of the experimental work detailed above was done by Mr. Frank Wright, assistant in the laboratory of physiological chemistry, and Mr. Charles Ericson, to whom my thanks are due.

NORTHWESTERN UNIVERSITY,
CHICAGO, April 24, 1900.

AN EXAMINATION OF BROWN AND TAYLOR'S OFFICIAL METHOD OF IDENTIFYING BUTTER.

BY JOHN A. HUMMEL.

Received March 7, 1900.

THE recent great increase in the production of renovated butter, due probably to the improvements in the method of its manufacture and the prevailing high price of creamery butter, has necessitated, in several states, the enactment of laws regulating its sale. Since the enactment of these laws there has been some discussion as to reliable methods for its identification. The fact that the chemical properties and the proximate composition of samples examined are entirely within the range of normal butter, has directed attention to its physical properties more particularly its behavior with polarized light. As is already well-known, in the manufacture of renovated butter, the butter-fat is melted and then cooled rather rapidly in a stream of cold water. This melting and rapid cooling induces a semicrystallization of

the fat. It was thought that this fact might be taken advantage of and would at least deserve some study. For the experiments carried on in this laboratory fifteen samples of renovated butter secured from factories in St. Paul, Minneapolis and Duluth were used; as many samples of undoubted normal butter from Minnesota creameries and dairies were also examined. The preparations for the microscopic examination were made by simply placing a small bit of the butter on a glass slide and pressing it into a thin film with a cover-glass. The preparation was then immediately examined with a polarizing microscope giving a magnification of 120 to 150 diameters. A selenite plate was placed between the slide and the lower nicol. In every case the normal butters gave with the selenite which was used a uniformly blue colored field, showing the entire absence of fat crystals. The renovated butters on the other hand gave a blue field mottled with yellow; this mottled appearance varied slightly in intensity but was very marked and distinctive in every case. The accompanying photomicrographs are from representative preparations and are believed to be fair averages. In making these photomicrographs the selenite was not used, the thin film of butter being simply placed between crossed nicols. The source of light was a 16 candle-power incandescent electric light, and an exposure of fifteen minutes was necessary for the normal butter, eleven minutes for the renovated butter, and eight minutes for the oleomargarine, using plates of medium rapidity.

The writer is well aware of the severe criticism directed against the method proposed by Dr. J. Campbell Brown, and later by Dr. Taylor, for identifying adulterated butter by means of the microscope and polarized light, yet he has found a microscopic examination of great value, and believes it to be one of the best methods for the identification of renovated butter, as found upon the Minnesota markets at least. In the course of work in this laboratory 247 samples of butter have been subjected to a microscopic examination as outlined above and out of this number 58 showed conclusive evidence of having been melted and cooled as in the process of renovating. A majority of those samples which could be traced to the manufacturers were admitted as being renovated and some fines have been imposed.

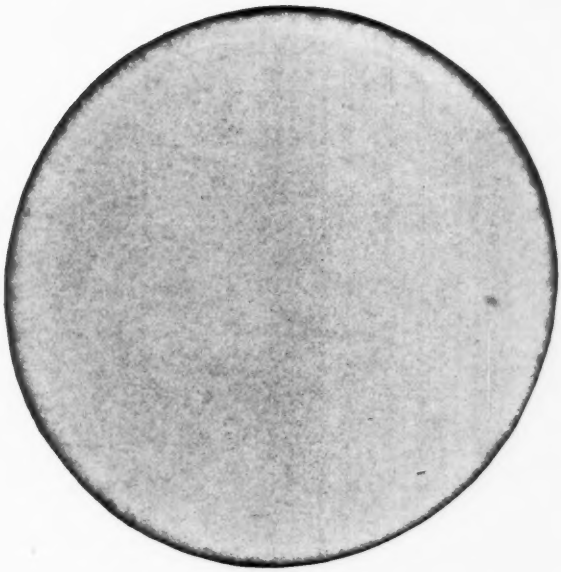

Fig. 1.—Normal Butter.

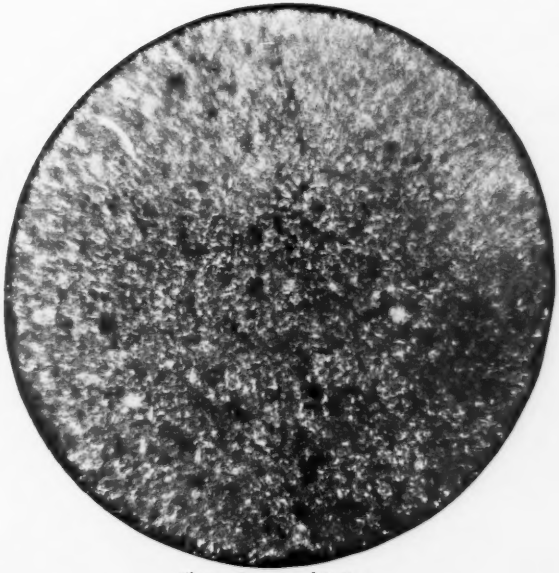

Fig. 2.—Renovated Butter.

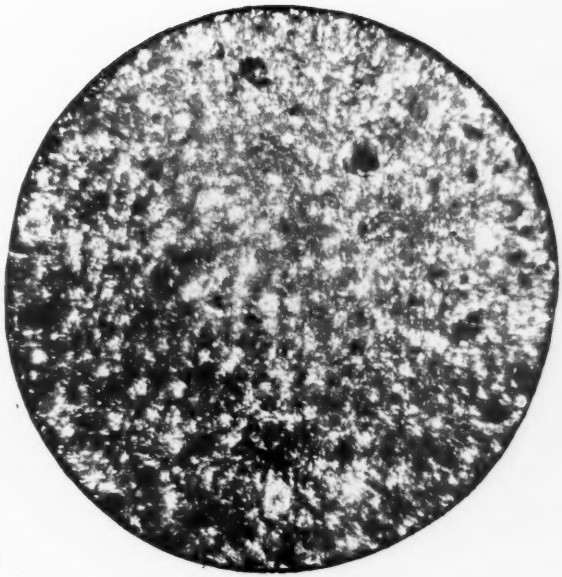

Fig. 3.—Oleomargarine.

In the examination of these samples the greatest reliance was placed in every case on the microscopic appearance, though other tests were also used.

LABORATORY OF THE MINNESOTA STATE DAIRY AND FOOD COMMISSION.

[FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, PATHOLOGICAL INSTITUTE OF THE N. Y. STATE HOSPITALS.]

ON THE PREPARATION OF NUCLEIC ACIDS.[1]

BY P. A. LEVENE.

Received April 14, 1900.

THE various modifications of Altman's and Kossel's methods of obtaining nucleic acid, which have appeared during the last year clearly demonstrate two facts: first, that the old methods were unsatisfactory; and secondly, that a thorough knowledge of the nucleo compounds is of ever-increasing importance to all investigators in the field of the chemistry of the cell.

All the new methods published in recent years are based on two properties of the nucleic acids; namely, their solubility in acetates and their resistance to dilute alkalies on heating. Both properties were observed only by the authors of the new methods. The improvement recently advocated by Schmiedeberg was based on the idea of removing (by means of copper salts) the proteid material which combines with the nucleic acids, to form the nucleo-compounds occurring in cells and tissues.

One of the properties of the nucleic acids described by the more recent investigators, namely their resistance to heating with alkalies, stands in contradiction to the observations of the older workers. Thus Miescher considered it requisite that the material which was being treated for nuclein should be kept at a very low temperature during every phase of its preparation. Kossel has succeeded in decomposing the ordinary nucleic acid of the thymus into thymic acid by heating it in water on the water-bath.

Neuman, advocating the heating method with alkalies, states that by this method more than one acid is generally obtained. From the fact that different proportions of the acids vary with the duration of heating, he draws the conclusion that the three acids are modifications of the one occurring in the tissue.

[1] Read before the New York Section of the American Chemical Society, April 6, 1900.

Hammarsten and Bang, who have applied the method of heating with alkalies for obtaining their guanilic acid, obtained a substance with properties differing greatly from those of all the other acids hitherto described.

Thus from the statements of the investigators advocating the "hot" method it may be admitted that the new methods, being an improvement over the old ones, they may be still further perfected, because they do change somewhat the original character of the substance.

In addition to this, I would like to add that to some nucleo-compounds the new method cannot be applied at all, as their decomposition on heating with very dilute alkalies takes place in so short a time that it is impossible to obtain any satisfactory yield by that method. I refer to the compounds known as para-nucleo-compounds.

A uniform method for obtaining all the nucleic acids is, however, most desirable. During the last two years I have been engaged in the study of different nucleo-compounds, and I have used, with satisfactory results, a method differing from those used by other authors. Fresh tissues, nucleo-proteid, as well as para-nucleo-proteids are treated with a strong solution of alkalies (5 per cent. sodium hydroxide or 8 per cent. ammonia), and allowed to stand in a cool place one to two hours. This solution or mixture is then gradually and slowly neutralized with acetic acid, care being taken not to add too much acid at a time, so that the temperature of the solution does not rise too high. It is advisable to keep the solution in a cooling mixture or to add ice to the alkaline mixture itself. When the mixture remains only slightly alkaline a saturated solution of picric acid is added until the mixture becomes neutral or nearly neutral (about 75 cc. of the picric acid to 1 liter of the mixture is generally sufficient); more acetic acid is added until the mixture is rendered strongly acid and then allowed to stand for some time, filtered, and to the filtrate is added 95 per cent. alcohol until the latter ceases to form a precipitate. This precipitate is nucleic acid. The picric acid is added in order to remove the proteid material. On neutralization with acetic acid a sufficient quantity of acetates is formed to enable the precipitation of all the nucleic acid by means of alcohol. (In the absence of acetates nucleic acid cannot be pre-

cipitated by alcohol.) By this method I have treated ovovitellin, ichtulin of cod-fish eggs, cod-fish sperm, pancreas and bacillus tuberculosis.

The very crude acid obtained from the ovovitellin contained 9.65 per cent. of phosphorus; three other samples purified contained, 10.02, 9.95, and 9.79; a copper salt contained, P, 8.57, and Cu, 12.36 per cent.; for the free acid, P = 9.78 per cent. The acid obtained from the same vitellin by Milroy in Kossel's laboratory varied in its contents of posphorus from 7.51 to 7.94 per cent. The acid obtained from ichtulin contained 8.46 per cent. of phosphorus. Walter, who studied the chemical nature of ichtulin in Kossel's laboratory, failed to obtain from it a substance similar to nucleic acid. The acid obtained from the cod-fish sperm was biuret free after the first precipitation and contained 8.65 per cent. of phosphorus. From the pancreas an acid was obtained with the same solubility as the other nucleic acids, while the substance described by Bang as guanilic acid differed in that respect from the other nucleic acids. From the bacillus tuberculosis the acid was also obtained biuret free after the first precipitation.

The study of the chemical properties of all the above-mentioned acids is now in progress.

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[CONTRIBUTIONS FROM THE HAVEMEYER LABORATORIES OF COLUMBIA UNIVERSITY, NO. 25.]

A CHROMIUM CELL FOR THE RECTIFICATION OF ALTERNATING CURRENTS.

BY J. LIVINGSTON R. MORGAN AND W. A. DUFF.

Received April 17, 1900.

MUCH attention has been given of late to aluminum rectifying cells. These consist of a platinum and an aluminum electrode in a solution of sulphuric acid or potash alum. When

the current is applied to such a cell, the aluminum electrode being the anode, the aluminum plate becomes covered with a highly resistant film which prevents, to a great extent, the further passage of the current in this direction. In the opposite direction however, *i. e.*, from platinum to aluminum through the liquid, the resistance of the electrolyte alone is encountered. If an alternating current is applied to such a cell, keeping the voltage down below a certain minimum point, an interrupted current, made up of the half waves of the alternating one, is the result. If the alternating voltage is increased gradually a point is finally reached at which the resistance of the film is overcome and the latter broken down after which the alternating current goes through unchanged. Up to this breaking-down point the current becomes less and less asymmetrical; *i. e.*, the opposite half waves become more and more alike in size. The minimum electromotive force which will cause the film to break down has been found by us as well as by others to be about 26 volts.

Our experimental work on this subject was done in the early part of 1899, but as it was not published at the time most of our results have since been found and published by others. The substitution of chromium for aluminum, however, has not yet been reported so that we propose to give our results very briefly here. At the time we did not expect to find any connection with the work of Hittorf[1] on chromium, but since such a one has been found we deem the result worthy of a short description for its bearing upon the general behavior of metallic chromium. Since our work was suspended in April, 1899, the results are fragmentary but we think of their kind, conclusive.

The electrolyte used throughout was 10 per cent. sulphuric acid. The chromium electrode consisted of a fragment of the chemically pure metal[2] fused into a glass tube containing mercury by which the connection was made. The platinum electrode was made of a strip of foil. The apparatus consists simply of a graded source of direct current, the amount going through the cell being measured by a low reading ammeter (0—5). A reversing switch is also used by which either the chromium or platinum may be made the anode. If the platinum

[1] *Ztschr. phys. Chem.*, 25, 729, and 30, p. 481.

[2] Eimer and Amend.

is first made the anode, the solution becomes yellow, and hydrogen gas is given off at the chromium electrode. If the chromium is now made the anode we find that no current goes through the cell, while when the platinium is the anode the current passes freely. We have then an asymmetrical resistance. *Using the chromium as anode and increasing very gradually the impressed electromotive force it is possible to stop any current entirely, as far as readings on the ammeter are concerned, until the pressure of about 75 volts is reached.* It is not difficult to stop this amount after the cell has once stopped it, but in starting, if the increase is made too rapidly, the current will force its way through. Of course, with the platinum as anode the resistance offered is only that of the sulphuric acid, so that the current is rectified with great efficiency.

When the increase in the electromotive force is made so rapidly that the current goes through from chromium to platinum, or after the cell breaks down by the application of more than 75 volts, a very peculiar result is obtained, and it is by this that Hittorf's results for the "active" and "inactive" states of chromium are confirmed. *Unlike the aluminum cell after it has broken down the chromium cell is still an asymmetrical resistance, but only when the platinum plate is made the anode. With chromium as the anode the current now passes freely.*

If in this condition the cell is broken down by the application of too high an electromotive force to the platinum anode another reversal takes place so that about 75 volts can again be stopped using the chromium as anode. The electromotive force necessary to cause this second reversal does not seem to be fixed, but the reversal always takes place. This change in the behavior of chromium is doubtless due just as were Hittorf's to the change from the "active" to the "inactive" state, and the change can be well shown experimentally in this way.

One other thing, we think, is proved by this work. Since chromium and aluminum act very much alike chemically, we should expect that they would both act asymmetrically in a cell and for the same reason. Aluminum has been supposed to act by a resistant layer of an oxid[1] and since chromium, when used in the same way, causes a reversal of the asymmetrical effect and

[1] Norden: *Ztschr. Elektrochem.*, 6, 159 and 188.

according to Hittorf its "active" and "inactive" states are not caused by resistant films, hence the asymmetrical effect of aluminum can only be caused by these; so that the theory of the action of the aluminum cell, as due to the formation of a resistant film, is confirmed.

REMARKS ON SOME METHODS OF DETERMINING CARBON IN STEEL.[1]

BY GEORGE AUCHY.

Received April 25, 1900.

IN Blair's "Chemical Analysis of Iron"[2] it is stated that combined carbon in steel can be determined by loss upon ignition, 70 per cent. of the loss representing the carbon. As Blair gives the percentage of carbon in the carbon sponge as "about" 70 per cent., and as the method is not in general use, it may be inferred that it is known to be inexact. Mr. George Dougherty, in the *Iron Age* for May 11, 1899, proposes a method which is identical except that the carbon sponge is washed four times with hot nitric acid (sp. gr. 1.13) to dissolve out copper and copper sulphide. It may be questioned whether this procedure makes the method an accurate one as Mr. Dougherty's article does not throw much light on this point (only one result being given). Some experiments were made by the writer, which showed that, on the contrary, the washing with hot nitric acid is itself a source of error in one particular, and a possible source of error in another. 1. The hot nitrc acid dissolves some of the carbon, and the amount dissolved varies with different steels. 2. The carbon sponge absorbs and retains a portion of the nitric acid, and this absorption is possibly not uniform in all cases. That the washing with hot nitric acid (sp. gr. 1.13) four times dissolves out carbon, and in varying amount, is shown in the following results, all from different steels:

[1] Read at the February Meeting of the Philadelphia Section.

[2] Blair's "Analysis of Iron," second edition, p. 151.

By combustion as usual. Per cent.	By combustion but washed with hot nitric acid. Per cent.	Percentage of loss	Carbon sponge boiled up or digested with hot nitric acid.
1.078	1.03	4.45	....
0.76	0.76	0.00	...
0.715	0.68	4.90	....
0.72	0.697	3.20	....
0.81	0.775	4.32	0.628 (Boiled up.)
0.665	....	....	0.62 (Digested one-fourth hr.)
0.665	....	....	0.56 (" two hours.)

Eleven other similar results were obtained in which the lowest percentage of loss was 2.15, and highest 5.74. On digesting a carbon sponge with hot nitric acid (sp. gr. 1.13), and filtering the filtrate after boiling to expel lower oxides of nitrogen, decolorized permanganate solution.

That the carbon sponge, when washed with hot nitric acid (sp. gr. 1.13), absorbs and retains nitric acid, is indicated by the following results:

No.	Weight of carbon sponge. Washed as usual. Gram.	Weight of carbon sponge. Washed twice with hot hydrochloric acid, six times with hot water, four times with hot nitric acid, and lastly ten times with hot water. Gram.
723	0.0856	0.0955
691	0.0583	0.0647
	0.0583	
723	0.0856	0.0880
739	0.0654	0.0747
740	0.0674	0.0742
BG2	0.0603	0.0750
737	0.0679	0.0740

No effort was made to ascertain whether the absorption was uniform in all cases. Instead the following determinations were made with a view to ascertaining the average percentage of carbon in the carbon sponge[1] and also at the same time the range of error in the process.

[1] Mr. Dougherty gives the percentage as 0.6750. This does not represent the absolute percentage of carbon in the nitric acid-washed carbon sponge, but the absolute percentage, plus the loss of weight, in terms of carbon, suffered by the paper disk in being washed by acid liquids, plus the loss in weight through the solubility of the carbon in the hot nitric acid wash. Nor does the writer's average factor (0.6000) represent the absolute percentage of carbon in the carbon sponge, but the percentage plus the loss by washing with hot nitric acid. The writer's filtrations were made through asbestos.

No.	1012	1013	1014	1015	1016	1017	Standard
Factor	0.6319 0.6333	0.6415 0.5845	0.5950 0.5898	0.5698 0.5897	0.5976	0.5575	0.6044 Average 0.6000

From the above it is seen that in a 0.60 per cent. carbon steel, results by this method might vary from 0.557 per cent. to 0.641 per cent. carbon, taking 0.6000 to be the factor. In the above tests only two washings with hot nitric acid (sp. gr. 1.13) in each case were made, in the lower row of tests, and three in the upper row to reduce the error from this cause as much as possible. The steels used were Swedish band saws containing not over 0.008 per cent. sulphur. If Meineke is right in considering the sulphur of the steel to be present in the carbon sponge in the free condition,[1] another source of error must be counted against the process when used for high sulphur steels.

THE USUAL COMBUSTION METHOD.

Professor D. H. Browne,[2] in describing the apparatus for the moist combustion method, says: "It is very necessary to have a small U-tube containing $CaCl_2$ in one leg and soda-lime in the other attached to the potash bulbs and weighed with them. The small straight tube designed to contain $CaCl_2$ attached to the potash bulb is not sufficient to prevent the escape of a slight quantity of water from the potash solution." Mr. A. A. Blair, in using the dry method, found that the calcium chloride tube did not absorb all the moisture drawn over from the potash bulb.[3] Mr. J. M. Camp, of Carnegie Steel Co., considers it necessary to have a weighed potash bulb containing strong sulphuric acid attached to the calcium chloride prolong.[4] The writer of this article several years ago described to this Society some experiences of this kind in using the wet method of combustion. The carbon dioxide was not completely absorbed by the potash solution (sp. gr. 1.27), and the moisture not completely retained by the prolong and drying train; the amount of moisture not retained by the drying train and passing into the potash bulb amounting to about 0.065 per cent. in terms of carbon with two sets of bulbs and prolongs in use, was 0.045 per cent. for the first set usually, and 0.02 per cent. for the second, and a correction to this amount

[1] *Ztschr. angew. Chem.* (1888), 376.
[2] *J. Anal. Appl. Chem.*, **5**, 338.
[3] *Ibid.*, **5**, 128.
[4] "Methods of Iron Analysis."

had to be made in each result. Professor Arnold also in his experience found a correction of 0.045 per cent. (using one bulb) to be necesssary.[1] Professor Chas. F. Mabery found a loss of moisture and suspected a loss of carbon dioxide.[2] This seems to be sufficient evidence that the usual drying and absorbing agencies are inefficient. On the other hand, many chemists using both methods of combustion, have, like Dr. Drown, satisfied themselves by actual trial of the efficiency of their apparatus in this respect. The writer himself upon changing to the dry method found no further difficulty of this kind of any great consequence[3] except at first due to the copper oxide being packed into the preheating furnace too tightly, and aspiration being made by the pump. Mr. Buck, of the Bethlehem Steel Co., and Mr. Sargent, of the Carpenter Steel Works (the former with a Shimer apparatus, the latter with a modified oxygen apparatus), use extremely rapid rates of aspiration (allowing only twenty minutes for a combustion) without loss, either, of carbon dioxide or of moisture. Yet it is certain that those of us who have experienced a lack of efficiency of this sort have not simply dreamed or imagined it. What, then, is the reason that the potash solution sometimes fails to absorb all the carbon dioxide, and the calcium chloride or strong sulphuric acid fails to absorb all the moisture? Some recent experiments by the writer suggest an explanation and indicate a source of error, which though very simple may easily be overlooked. This source of error apparently lies in the fact that when there is undue resistance to the passage of gas through the apparatus for any reason, usually because of the calcium chloride or copper oxide having become clogged up in use, or having been packed in too tightly, or too finely divided in the first place, the gas then bubbles through the potash solution and the strong sulphuric acid, and passes through the calcium chloride not evenly and regularly but the reverse, and although precisely the same volume of gas may pass through as in normal conditions, the contact of gas with drying and absorbing materials is obviously less than normal.

[1] "Steel Works Analysis." Professor Arnold thinks this due to something generated in the combustion flask, but the writer obtained the same result by simply aspirating air through the apparatus for the same length of time.

[2] This Journal. **20**, 510.

[3] Some moisture escaped from the prolong after being in use a number of tests, but this is now believed to be due to a cause that will appear later on.

This irregularity of action and accompanying loss may be due simply or mainly to the size and shape of the potash bulb, or may have its origin in the oxygen cylinder; but whatever the cause it seems to be a fact that when the gas does not pass freely and easily through the apparatus, and does not bubble evenly and regularly through the bulbs, loss ensues if a rapid or moderately rapid flow be allowed.

The experiments were as follows:

No	Prolong clogged up. Carbon. Per cent.	Prolong in good condition. Carbon. Per cent.
1012	0.715	0.76
1013	0.68	0.716
1015	0.705	0.718

In the last experiment with clogged prolong the rate of speed of gas flow was diminished. With fresh calcium chloride in the prolong the following tests were made. The loss was believed to be due to the resistance offered to the passage of the gas by the 3 inches of rather closely packed copper oxide in the combustion tube.

Steel "S," usual rate. Carbon. Per cent.	Steel "S," slower rate. Carbon. Per cent.
1.053, 1.066, 1.06	1.078, 1.076, 1.077
1.067, 1.07, 1.065	....

The first result under "usual rate" is not the writer's, but that of another chemist, and may perhaps be taken as an indication of some impediment to the flow of gas in his apparatus also. These results and the preceding ones seem to show that it is highly important to have the gas pass through the apparatus freely and easily if it is desired to maintain a rapid or moderately rapid rate of gas flow. Using another apparatus (a Shimer apparatus) in which the flow of gas is very free and unobstructed, no higher results could be obtained by using a slow rate than by using a rapid rate of gas flow. With the first-mentioned apparatus the writer now uses a rate of one bubble per second through the first or purifying bulb for one-half hour followed by ten minutes of a faster rate, and believes this rate slow enough not only to counteract the density of the copper oxide but also to allow for any clogging up of the calcium chloride that may occur; but this slow rate is, of course, impracticable where many combustions have to be crowded into

a day, or when a combustion must be made in the very shortest possible time; when a rapid rate is employed it would seem imperative to be sure that the gas passes freely and easily through the apparatus, and bubbles regularly and evenly through the potash bulbs. Calcium chloride sometimes clogs up and strong sulphuric acid seems preferable. The writer believes that for the calcium chloride prolong, one could profitably substitute diminutive bulbs for strong sulphuric acid of the Geissler type but constructed to hang instead of to stand so as to allow of being weighed along with the potash bulbs by being suspended from the hook of the balance.

These experiments perhaps throw some light on the cause of the losses of carbon dioxide and moisture experienced by the writer in using the wet method of combustion. Possibly these losses were caused by some stoppage in the apparatus or irregularity of gas flow. As a matter of fact the aspiration (which took place from beginning to end of the process) was made by a filter-pump which the writer has frequently observed does not exert an even and regular suction. Possibly all such losses are attributable (the drying and absorbing materials not being too long in use) to some such cause—perhaps a clogging up somewhere in the apparatus causing an irregular and jerky gas flow; or perhaps too much apparatus, causing, by its resistance, an uneven flow.

Dr. P. W. Shimer has greatly simplified the dry combustion apparatus and his apparatus will, without doubt, eventually altogether displace the old form. He also makes a notable change in the direction of greater simplicity—in the drying and purifying train. A train which vies with Shimer's for simplicity is that of Prof. Arnold's described in his book, "Steel Works Analysis," but in other respects there is a remarkable difference between the two. Shimer's train: cold water for HCl and Cl; calcium chloride for drying; Arnold's train: chromic acid solution for SO_2; concentrated sulphuric acid for drying. It is seen that the difference is fundamental. The former is based upon the belief generally prevalent among American chemists that hydrochloric acid or chlorine, if present in the carbon sponge, is not absorbed by the copper oxide but passes out of the combustion tube, while sulphur dioxide is believed to be converted into sulphur trioxide in the pres-

ence of the glowing copper oxide and absorbed by it. Prof. Arnold's belief is the exact opposite of this; namely, that hydrochloric acid and chlorine are absorbed by the copper oxide of the combustion tube (p.40), and that sulphur dioxide escapes therefrom unchanged. Which theory is correct? That hydrochloric acid, if present in the carbon sponge, is not absorbed by the copper oxide, and does pass out of the combustion tube may easily be proved by testing the water of the Shimer absorption tube after a number of determinations have been made. On the other hand, as proof that sulphur dioxide passes out of the combustion tube, Arnold mentions an experiment in which potassium permanganate solution, placed after the combustion tube and connected with it, became decolorized by the gas. The writer finds that in using Arnold's chromic acid solution immediately following the combustion tube, as he directs, the chromic acid becomes entirely reduced, and the color of the solution changed to green after a considerable number of combustions have been made. But that this reduction is effected by hydrochloric acid,[1] and not by sulphur dioxide, is believed to be proved by the fact that when a bulb containing cold water is interposed between the combustion tube and the chromic acid bulb, the color of the chromic acid solution does not change; nor does the water of the bulb preceding show any trace of sulphur dioxide when tested with permanganate solution. If traces of the gas ever escape absorption by the copper oxide, Shimer's cold water tube would seem to be an ample safeguard. But as a further precaution, concentrated sulphuric acid, saturated with chromic acid, may be substituted for calcium chloride in the train.

The fact observed in the examination of Dougherty's method, of the carbon sponge being slightly soluble in hot nitric acid, suggested the suspicion that it might also be soluble in hot acid double chloride solution to some extent. The following tests were made on the same sample of steel:

Hot double chloride solution. Carbon. Per cent.	Cold double choloride solution. Carbon. Per cent.
1.052, 1.050, 1.06	1.076, 1.078, 1.077

In these tests the double chloride solution was heated (after

1 Five drops (and probably less) of hydrochloric acid will completely reduce 10 cc. Arnold's chromic acid solution in the cold, standing over night.

solution of precipitated copper was nearly complete) for an hour or more, giving a much severer test of the solubility of the carbon than would occur in actual practice. It may therefore be inferred that the error from this cause in actual practice is very slight or in some cases nothing, for as the second result of the first table of results in this article shows that the carbonaceous residues from some steels are unacted on by hot nitric acid, so it is probable that such residues resist the slightly solvent action of hot double chloride solution. On the other hand, some residues may be much more soluble in hot double chloride solution than those of the above table. The point brought out by these results is merely that heating the double chloride solution of the drillings is attended with some risk of loss.

The carbon residue of the first table, that was not acted on by hot nitric acid was from a saw—a much-worked product. The carbon residues from such finished products seem different from those of ingot steels inasmuch as they give much darker shades to nitric acid in the color test than do the latter; thus, for instance, a 1.07 per cent. carbon steel in a finished saw gave 1.27 per cent. by the color test using a hammered slab as standard.

In washing the carbonaceous residue with hydrochloric acid it is perhaps safer to have the acid cold.

In carbon determinations, the error from moisture condensation in damp weather is well known. In a communication to the Society nearly two years ago the writer gave the results of experiments made in very damp weather which indicated the error from this cause to be usually 0.02 per cent. or 0.03 per cent., and occasionally as high as 0.05 and 0.06 per cent. When these experiments were made it was the writer's habit to have the balance-room door open; this door led into a small ante-room the door of which, at that time, also stood habitually open on account of warm weather, so that the outside air had access to the balance-room. For a long time back, however, the balance-room door has been kept closed, at least during the progress of combustions, and no such excessive condensation as 0.05 per cent. and 0.06 per cent. have occurred, using extra bulbs as an indicator. It is therefore believed to be well to keep the balance-room closed, at least in warm weather when adjoining parts of the laboratory are open. This is, of course, not an absolute

preventive. Moisture condensations equivalent to something under 0.02 per cent. carbon may easily occur.

THE COLOR METHOD.

The color method for high carbon steels is generally regarded with much distrust. Mr. Wm. Metcalf in his book, "Steel", says: "For in high carbons the color test in the best hands is only the wildest guess work." It is the writer's experience that the same drillings, even when dissolved at the same time, frequently do not give the same shade, and that by performing the test in the usual way, results as high as 0.10 per cent., or even higher, wide of the truth are often obtained. If the usual method be elaborated as follows, results not further from the truth than 0.03 per cent. (usually 0.01 or 0.02 per cent.), or at most 0.04 per cent. can uniformly be obtained: Duplicate tests of standard and samples are weighed out. Besides the standard, duplicate tests of two other steels in which the carbon has been accurately determined by combustion are also weighed out. In the absence of such steels, six samples of the standard are taken. When the color comparisons are made, the two steels of known carbon content are first tested, and from these tests is averaged a correction to be applied to all the results subsequently obtained. When these tests are finished the second sample of the standard is taken, the correction found as before, and the testing of the samples duplicated, and the average of these duplicates taken. These precautions are simple and obvious but are of no avail in the presence of a difficulty which, although it happens very rarely, still does happen. This difficulty is a carbon color showing a *much* deeper shade placed to the left of the standard than to the right of it, and requiring a further dilution equivalent to about 0.10 per cent. carbon to make the shade to coincide with the standard, the color when placed to the right showing no change by this dilution, and still agreeing with the standard. The result is then too high by just the amount represented by this extra dilution, or about 0.10 per cent., and it is necessary, therefore, when this abnormal shade is noticed, to discontinue the dilution and to take the reading when the color at the right coincides, ignoring the left-hand shade altogether.

No	As usual. Carbon. Per cent.	Left-hand shade ignored. Carbon. Per cent.	Combustion. Carbon. Per cent.
A4.....	0.96 0.99 0.99	0.89	0.88
A6.....	0.955 0.965	0.85	0.85 0.85

LABORATORY OF HENRY DISSTON AND SON'S STEEL WORKS,
PHILADELPHIA, PA.

[CONTRIBUTION FROM THE LABORATORY OF ANALYTICAL AND APPLIED CHEMISTRY, UNIVERSITY OF MICHIGAN.]

A BURETTE FOR ACCURATE GAS ANALYSIS.

BY ALFRED H. WHITE.

Received April 27, 1900.

EVERYONE who has attempted to make accurate analyses of gases with the usual Hempel apparatus for technical gas analysis, knows there are many unavoidable sources of error, slight in themselves, but enough to make an exasperatingly large total at the end of a long analysis. The apparatus to be described was devised in the effort to eliminate some of these sources of inaccuracy. It is a modification of the Hempel burette with Petterson correction tube, the alterations being in the design of stop-cock and the position and form of the manometer, besides a change in the shape of the graduated tube to permit a more accurate reading of the gas volume. It has been used in its present form for the past year in this laboratory, and has been found to fulfil its purpose.

Some of the errors incident to analyses as usually conducted may be avoided by well-known precautions. If mercury instead of water is used as the liquid in the burette, errors due to solubility of the gas in the burette liquid and errors in reading due to water adhering to the walls of the burette, vanish. Errors due to solubility of gas in the reagent of the absorption pipette may be minimized by the use of a small quantity of the reagent in a pipette otherwise filled with mercury and may be greatly lessened when using an ordinary pipette by saturating the absorbing liquid with gas like that to be analyzed. Errors due to diffusion of gas through the liquid in the pipette are much more important than is usually believed, but may be obviated for most reagents by the use of a few cubic centimeters of mercury,

forming a trap at the bottom of the pipette. The errors which this paper aims to discuss and in part to remedy are those due to: Change in temperature and barometric pressure during an analysis; inclusion of air or leakage of gas while making connection with pipettes; and inaccuracy in reading gas volume.

Gas burettes in which the gas volume is unaffected by change of temperature and pressure have been devised and are well known. The Petterson correction tube as modified by Hempel, Drehschmidt, and others, consists of a glass tube of about the same dimensions as the burette, sealed at its lower end and with its upper end connected to one arm of a manometer whose other arm connects through a three-way stop-cock with the gas burette. This correction tube is enclosed in the same water-jacket as the burette, and hence the gas volume in it is affected by changes of temperature to just the same extent as the gas in the burette. When the liquid stands at the same height in both arms of the manometer, the pressure in the burette is the same as in the correction tube and therefore a constant, as the correction tube is sealed at its lower end. If the gas volume is read under these conditions it will be independent of changes in external temperature and pressure. There are several objections to the Hempel form of apparatus. The two rubber connections of the manometer may allow leakage of gas and consequent change in the supposedly constant pressure of the correction tube. Second, in drawing the gas out of the manometer into the burette there is a pronounced tendency on the part of the column of liquid to break when it starts down hill, especially at the rubber connection, with the result that some of it becomes carried into the burette with consequent change in the pressure indicated by the manometer. Figures 1 and 2 show the arrangement designed to overcome this difficulty. There is but one rubber connection in the manometer, and that is placed so that only mercury and never gas is brought in contact with it, thus rendering a leak with consequent change in pressure of the correction tube impossible. Communication with the manometer is established by turning stop-cock to position in Fig. 4. By placing the manometer below the stop-cock the further advantage is gained that in drawing the gas out of the manometer the mercury travels but a short distance and up hill only, meets with no bends

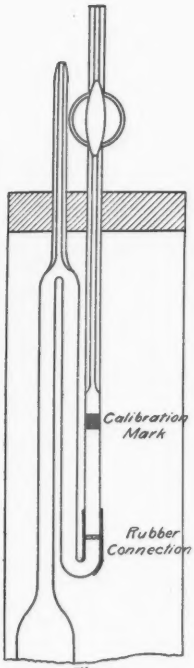


Fig. 1.

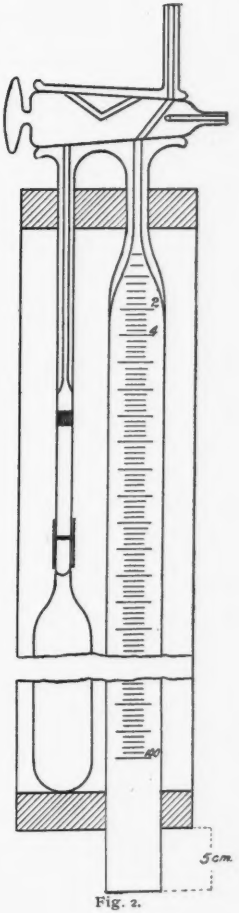


Fig. 2.

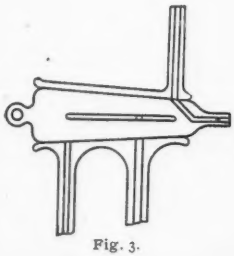


Fig. 3.

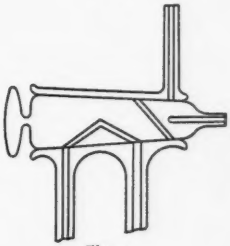


Fig. 4.

or inequalities in the tubing, and so may be drawn with great exactness to the stop-cock, drawing out all the gas without losing any of the mercury. The apparatus is all enclosed in the water-jacket and therefore all the same temperature, and is less cumbrous and apt to get broken than in other forms. The capillary tube projecting into the air behind the stop-cock as shown in Fig. 1 is to be sealed, under observed barometric pressure if desired, when the apparatus is first set up. The gas volume may be read when the mercury is at the same height in the two arms of the manometer and may then be reduced to standard conditions if desired. When this is not required, it is much more accurate to bring the mercury to a mark etched on the glass, or better tangent to the lower edge of a black metallic collar cemented around the manometer tube. In this case the gas will not be under the same pressure as that in the correction tube, but the variation will be a constant one and will cause no error. The connection between the burette and pipette is, as usual, made by a bent capillary tube and two rubber connections. Rubber connections, if properly made, introduce no appreciable error, but, because of their tendency to leak, form a serious menace to accuracy. Unfortunately no way has been found to dispense with them, and they are accepted by the author as a necessary evil. There is, however, an inherent source of error in the usual method of making the connection which the burette here presented completely obviates. With the apparatus ordinarily used, some of the gas under analysis will escape, or some unmeasured volume of air will be admitted to it whenever a connection is made. Perhaps usually both happen. The capillary tip of the burette, the connecting capillary, and the capillary tip of the absorption pipette, have a combined volume of about 0.5 cc. Although by various manipulative devices it is possible to restrict the error thus introduced from any individual operation to a fraction of this volume, it amounts in the course of a long analysis to a serious total. In the burette here presented it is completely eliminated.

By referring to Fig. 3, the stop-cock is shown turned in such a way that there is a clear passage from the capillary exit tube of the burette through the stop-cock to the outer air again. With the stop-cock in this position the pipette is to be connected

in the customary way to the burette but without any of the usual precautions to prevent inclusion of air. The liquid in the pipette is now blown up and through the capillary till it reaches the stop-cock. The capillary tube is thus completely filled with liquid. By turning the stop-cock to the position shown in Fig. 2 communication is established between the burette and pipette, and the gas may be passed over and absorbed as usual. After the absorption it is drawn over till the pipette-liquid just reaches the stop-cock, which is then turned back to the position in Fig. 3, allowing the liquid in the capillary of its own accord to siphon back into the pipette. The capillary is then to be rinsed out with a wash-bottle. In this way all the gas is each time drawn back into the burette for measurement. There is no gas lost in the capillary connecting tube, and no air is introduced, if the rubber connections have been properly made.

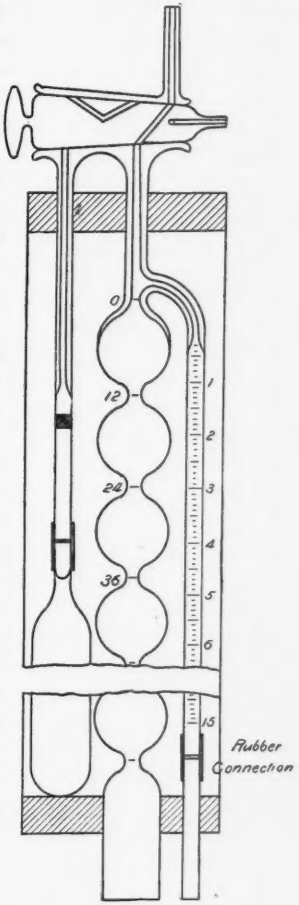


Fig. 5.

The gas volume in an ordinary gas burette may read with great accuracy with the assistance of a telescope, or some similar help, but it was desired to render such an adjunct unnecessary. The suggestion was taken from one of Bleier's[1] sketches. Fig. 5 shows schematically a gas burette with stop-cock and manometer as already described. The main body of the burette contains twelve bulbs, each of a capacity approximating 12 cc. A line is etched on each constriction and the capacity of the

[1] C. Bleier, "Ueber gasometrische Apparate." *Ber. d. chem. Ges.*, **31**, I, 238.

bulb between these marks is determined. Starting from the capillary above the top bulb a side arm springs, terminating in a small burette with total capacity of 15 cc. and graduated in 0.1 cc. Both these burette tubes connect at the bottom by means of heavy rubber tubes and a Y with a stop-cock on each arm, to a common leveling bottle. A screw clamp on each rubber tube serves for the exact adjustment of the mercury. To measure a gas, the stop-cock is placed in position in Fig. 3 and the mercury in the bulbed tube brought to the mark in one of the constricted portions by opening the proper stop-cock on the Y and raising or lowering the leveling bottle. When adjusted, the mercury is held in its proper position by closing the stop-cock on the Y. The stop-cock leading to the small burette tube is then opened and the gas brought to approximately atmospheric pressure by proper change in the mercury level. The three-way stop-cock at the top of the burette tube being now turned to position in Fig. 4, the burette is brought into connection with the manometer, which is properly set by further changing the level of the mercury in the small burette. The final adjustment in both burettes is made by the screw clamps on the rubber tubes. The volume of the gas will be read as x cc. in the bulbs $+ y$ cc. in side burette $+ z$ cc. in manometer. As there are these three readings to be made it is necessary that each be very accurate. Let us see how accurately this may be done. First, the mercury in the bulbed tube is to be brought to a specified mark in a tube of about 5 mm. internal diameter. By means of the screw clamp this may be done with such accuracy that the error is negligible. Second, the volume of gas in the side tube must be read. Each 0.1 cc. in this tube occupies a space of a little over 2.5 mm. and it is possible to interpolate 0.01 cc. with the eye with an error of less than 0.02 cc. Third, the mercury in the manometer must be brought to a definite mark with such exactness that the barometric pressure, under which the gas volume is read, shall be almost identical each time. A difference of 1 mm. of mercury pressure changes the gas volume 0.13 per cent., which on a volume of 100 cc. equals 0.13 cc., an error far too large. It was found impracticable to attain the required accuracy when it was attempted to bring the mercury to a mark etched on the glass. The best

device was found to be a band of thin, blackened copper, wrapped around the tube and cemented to the glass. It is possible to bring the mercury tangent to the lower surface of this with great exactness. In working with this burette the author is accustomed to make all readings in duplicate, readjusting at all points each time, and to repeat if the two differ from each other by more than 0.01 cc. Duplicates usually agree within this limit. The greatest difficulty found in manipulation is to draw the liquid from the pipette over exactly to the burette stop-cock and stop it there. If it gets into the burette, a bubble lodging in one of the capillary tubes frequently damps the sensitiveness of the manometer. If this happens the bubble may be shot out of its lodging place by compressing the rubber tube above the screw clamp. Such a bubble may also be carried into the manometer, where it will obscure the surface of the meniscus. To remedy this it is well to keep 2 or 3 mm. of water on the surface of the mercury in the manometer. This allows a perfectly sharp reading of the mercury meniscus below the water-level. The manometer should respond to a very slight movement of the screw clamp.

The advantages of this burette may be summarized as follows: It is a compact burette which, without reading-telescope or other accessories, allows the volume to be read with an error of less than 0.02 cc., compensates automatically for changes of temperature and pressure, and avoids completely all errors due to inclusion of air or loss of gas in making connections with the absorption pipettes. The disadvantages so far developed are chiefly those inherent in all forms of apparatus which possess a stop-cock and rubber connections. Both may leak; but on the other hand both may be kept so tight for limited periods of time as to introduce no measurable error.

Accurate apparatus is essential for accurate work and the author feels it but a just acknowledgment to thank Messrs. Greiner and Friedrichs for the care and skill with which they manufactured the burettes from his sketches.

ANN ARBOR, MICHIGAN,
April, 1900.

PREPARATION OF THE BLUE OXIDE OF MOLYBDENUM AND OF METALLIC MOLYBDENUM.

By Allen Rogers and F. H. Mitchell.

Received May 8, 1900.

BY the action of stannous chloride on an acid solution of a molybdate, a blue precipitate is produced. It was the investigation of this precipitate, that led to the following process: To prepare the blue precipitate, dissolve 50 grams of molybdenum trioxide in a sufficient quantity of ammonium hydroxide, to make a clear solution; to this add about the same quantity of water, make slightly acid with nitric acid, and then dilute with about five times its volume of water. To this solution add from 15 to 25 cc. of a 10 per cent. solution of stannous chloride. The very heavy precipitate which forms settles, and should be washed several times by siphoning off the supernatant liquid. These washings should be made as quickly as possible, as the blue precipitate becomes hydrated on standing, thus giving a deep blue solution.

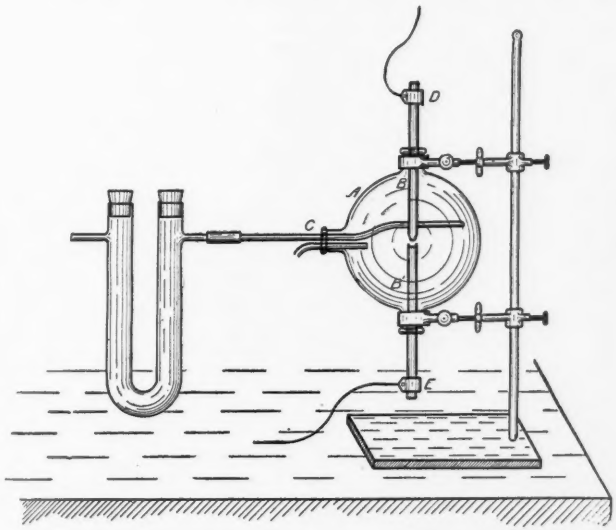
After washing, the precipitate is thrown upon a plaited filter, and allowed to drain. It is then removed from the paper, while moist, into an evaporating dish, placed in the oven and dried at 100° C. This precipitate seems to correspond to the oxide of molybdenum, Mo_3O_8.

Place some of this oxide in a porcelain boat, and insert into an ignition tube, pass in hydrogen for a few minutes, then apply heat from a Bunsen burner for one hour, allow to cool, weigh and heat again for fifteen minutes, and continue heating until the weight is constant. The reduction takes from one hour to one and one-half hours, and may be hastened by moistening the oxide with a few drops of water. The molybdenum as prepared in this way is an ashen gray powder. The blue oxide is reduced more readily than the trioxide, under the same condition. In the electric furnace the blue oxide is rapidly reduced.

The accompanying figure illustrates a device by which we have been able to reduce various substances, and is very convenient in working with a small quantity.

The apparatus consists of a three-necked flask, A; the carbons, B and B′, are held in position by asbestos plugs. B′ has a

cavity in the end, into which the substance to be reduced is inserted. At C is an inlet and an outlet tube, by means of which an atmosphere of hydrogen is obtained. Fill the cavity



in B′, remove the air, connect D and E with B and B′, by means of copper foil, and having the proper resistance, turn on the current, and allow to pass until the desired effect is produced. The oxide was reduced in forty seconds, and, prepared in this way, resembles the product of the electric furnace; that is, a dull steel gray mass, which is very hard.

UNIVERSITY OF MAINE, ORONO, ME.

SOD OIL, WOOL GREASE, AND DEGRAS.

BY ERASTUS HOPKINS.

Received May 14, 1900.

WHEN the tariff act of 1897, known as the Dingley bill, was made a law to govern the classification of imported articles, there appeared a paragraph in the act which, with an intent of definite designation, opened a question which became so involved that its final settlement was made only after litigation.

The paragraph is No. 279, which reads "....; wool grease, including that known commercially as degras or brown wool grease, one-half of one cent per pound."

The introduction of the term degras caused all the trouble, because the authorities framing the bill did not make the fact clear that the term degras is applied to oils and greases used by tanners without any special distinction.

For the question involved, research work was done in this laboratory on wool grease and sod oil, the results upon the latter being published in this Journal in 1899, p. 291.

As a result of the trial of the cases involved, sod oil was decided to be entitled to free entry as an oil used expressly for currying leather not specially provided for in the act, paragraph 568, and not similar to wool grease. Since that time I have been so often approached by people regarding the facts pertaining to the substances involved in the case that I thought the subject might be of wider interest than to the limited circle of my professional acquaintances.

Sod oil and wool grease have entirely different constitutions as well as characteristics, and hence should be easily distinguished apart.

I wish especially to call attention to the terms "wool grease," "degras," and "sod oil."

Wool grease is extracted from the wool of sheep. Sod oil is expressed or extracted from leather which has been curried by oils, particularly fish oils.

Sod oil has no relation to wool grease in its derivation, but is related to it in its use; that is, for the currying of leather. Sod oil contains a resinous substance (not a resin) known as degras former, which is characteristic of sod oil. No other oil or grease (and this includes wool grease, which is, scientifically speaking, an animal wax and not a grease at all) contains this degras former, which is therefore characteristic of sod oil.

Originally, sod oil was called degras. Later the term was made by the American oil trade and to a less extent by the English to embrace wool grease. The term degras has therefore come to embrace two substances, dissimilar in constitution, source, and chemical constants. Wool grease being called degras by the trade, caused the mention of degras in paragraph 279 of

the act of 1897—a commercial term by which wool grease is known, as it is also known under the name of brown grease. The point I wish to make is that the degras there mentioned means wool grease but it does not embrace sod oil which in no way resembles it except in use. From this it can be seen that the term degras which originally had a limited meaning has come to be applied indiscriminately to the two substances used for currying and stuffing leather, and was mentioned in paragraph 279 to make that paragraph stronger in covering wool grease.

Paragraph 279 mentions two substances, tallow and wool grease. Degras is not mentioned in that paragraph excepting as a wool grease degras. Brown grease is mentioned also in this same connection.

Paragraph 568 mentions "all other greases and oils used for stuffing leather." Here also is included a degras but this degras is not that specially mentioned in paragraph 279 which is a wool grease, but is the original degras, or sod oil made from oil expressed from leather, and therefore having no connection with a wool grease which comes from wool. Sod oil is the English name for the substance known to the French as degras or moëllon and the term degras is the original French name for this oil extracted from oiled chamois leather and has been borrowed by the English to be misapplied to wool grease.

The term sod oil is unknown in the French currying trade excepting as an English translation of their own word degras or moëllon, and the process of making degras might be said to have originated in France in the manufacture of chamois leather.

(CONTRIBUTION FROM THE CHEMICAL LABORATORY OF LAFAYETTE COLLEGE.)

A METHOD FOR THE DETERMINATION OF ZINC BY THE USE OF STANDARD THIOSULPHATE SOLUTION.[1]

BY RICHARD K. MEADE.

Received May 4, 1900.

AT the spring meeting of the Lehigh Valley Section, the author read a paper[2] upon a new volumetric method for magnesium. The method depended upon the precipitation of

1 Read at a meeting of the Lehigh Valley Section, January 18, 1900.

2 This Journal, **21**, 746.

the magnesium as magnesium ammonium arsenate, $Mg_2(NH_4)_2As_2O_8+H_2O$, and the determination of the arsenic in this precipitate by digestion with potassium iodide in a strongly acid solution, and titration of the liberated iodine with standard thiosulphate. When a solution of arsenic acid contains sufficient sulphuric or hydrochloric acid, the arsenic is quickly reduced by potassium iodide even in the cold from the higher to the lower state of oxidation. The reaction is expressed by the equation

$$As_2O_5 + 4KI + 4HCl = As_2O_3 + 4HCl + 2H_2O + 2I_2.$$

Zinc is usually determined as a pyrophosphate by precipitation as zinc ammonium phosphate and ignition of this latter. Since the method above outlined for magnesium was suggested by the analogy between the phosphates and arsenates, the thought also occurred to me to make use of a volumetric method for determining zinc, somewhat similar to that for magnesium, by precipitating the zinc as an arsenate instead of a phosphate and determining the arsenic in the precipitate by titration with standard thiosulphate solution. From the equation given above and the formula for the zinc ammonium arsenate, each cubic centimeter of decinormal sodium thiosulphate should be equivalent to 0.00327 gram of zinc.

The method as tried for zinc was as follows: Pure zinc oxide, ZnO, was dissolved in dilute hydrochloric acid, an excess of ammonia added, and then 50 cc. of a 10 per cent. solution of sodium arsenate. The solution, after dilution to about 750 cc., was next warmed and nitric acid added until a slight turbidity was produced when the addition of nitric acid was discontinued and acetic acid added in its place, 1 cc. at a time, until the solution reacted acid to test paper. The precipitate was changed from curdy and flocculent to heavy and granular by heating and stirring. After allowing the precipitate to settle it was filtered off and washed. A hole was next punctured in the filter-paper, the zinc ammonium arsenate washed into a beaker, and 50 to 60 cc. of dilute hydrochloric acid added. The paper and the beaker in which the precipitate was formed were washed with the dilute acid until the solution and washings measured 85 to 100 cc. when from 2 to 3 grams of potassium iodide were added to the solution, and after allowing this latter to stand in

the cold a few minutes, the standard thiosulphate was run in from a burette until the color of the solution faded from red-brown to pale straw color and then, drop by drop, until all color vanished. The addition of starch for an indicator of the end of the reaction was found unnecessary in both the determination of magnesium and zinc by this method. Below are the results:

	Zinc oxide taken. Gram.	Equivalent in Zinc. Gram.	Zinc found. Gram.
1	0.2490	0.2000	0.1998
2	0.2490	0.2000	0.1994
3	0.2490	0.2000	0.2002
4	0.1245	0.1000	0.1004
5	0.1245	0.1000	0.1002
6	0.1245	0.1000	0.0998

Since calcium and magnesium are precipitated from alkaline solutions by sodium arsenate while zinc from an acid one, the successful attempt was made to separate the latter from the former two by first making the solution strongly ammoniacal, adding sodium arsenate, filtering off the calcium and magnesium arsenates, then making the solution acid with nitric and acetic acids and filtering off and determining the zinc as described above. The completeness of the separation is shown by these results.

Zinc oxide taken. Gram.	Equivalent in zinc. Gram.	Calcium carbonate added. Gram.	Magnesium oxide added. Gram.	Zinc oxide found. Gram.
0.2490	0.2000	0.2000	0.1000	0.1996
0.2490	0.2000	0.2000	0.1000	0.1992
0.2490	0.2000	0.2000	0.1000	0.2004
0.0623	0.0500	0.2000	0.2000	0.0500
0.0623	0.0500	0.2000	0.2000	0.0502
0.0623	0.0500	0.2000	0.2000	0.0496

Unfortunately manganese can not be so easily separated from zinc. In the case of ores, etc., it may, however, readily be gotten rid of by treatment with potassium chlorate and nitric acid after the solution of the ore.

The determination of zinc in a sample of ore was tried by first throwing down the manganese as a dioxide with potassium chlorate and nitric acid and filtering, separating the iron and alumina by double ammonia precipitations, then adding a large excess of sodium arsenate and filtering off the calcium and magnesium arsenates formed, and finally precipitating the zinc

by addition of nitric and acetic acids. The iodine liberated by digesting this precipitate in an acid solution of potassium iodide was then titrated with standard thiosulphate and the zinc calculated from the reading on the burette. The time required to run four determinations, without any effort at speed, was about eight hours.

The analysis of the ore by careful gravimetric methods was

	Per cent.
Zinc oxide	37.58
Manganese monoxide	15.33
Ferric oxide	29.69
Calcium oxide	1.12
Magnesium oxide	0.77
Silicon dioxide	12.77
Carbon dioxide	1.23
Water	0.76
	99.25

Metallic zinc, 30.18 per cent.

By the volumetric method, outlined above, the results were

	Metallic zinc. Per cent.
1	30.00
2	29.98
3	30.04
4	29.98

LAFAYETTE COLLEGE, EASTON, PA.,
January 18, 1900.

NOTES.

The Alleged Adulteration of Milk with Brain-matter:[1]— In the 1898 report of the Dairy Commissioner of the State of New Jersey, page 50, one of the chemists acting for the Commissioner, refers in rather intemperate language to the statements made in some text-books, concerning brain-pulp as a possible adulteration of milk and asserts that such adulteration has never occurred. He quotes the statement of König[2] to the effect that such addition to milk involves practical knowledge and resources which would hardly be possessed by milk-producers or purveyors, but Dr. König has not foreseen

1 Read before the Philadelphia Section of the American Chemical Society, March 15, 1900.

2 *Nahrungs- u. Genussmittel*, **2**, 256.

the spread of education and the aid that such people may get from books and even from newspapers. We may, however, judge from König's statement that the German reports give no authentic instance of such addition, but we would be mistaken if we were to regard the allegation as on the same par with the popular fallacies about sand in sugar or chalk and water as a milk substitute. A consultation of trustworthy authorities will furnish some interesting data.

The earliest reference to the subject seems to have been in an anonymous article in the *Gazette d. Hôpitaux* (1841). Unfortunately the file of the *Gazette* at the College of Physicians lacks the numbers for 1841, but the general opinion of those who have consulted the article is that its statements are not worthy of credit. It appears to have been written in consequence of the agitation in France at that time over the enactment of laws regulating the sale of milk. The general subject of the composition of milk, its adulterations and the means of detecting them, had been discussed at great length by Quevenne in the *Ann. d. Hyg. Pub. et d. Med. Leg.* for 1841. In the same journal for 1842, many pages are given to the discussion of the adulteration of milk by brain-matter, the papers being by Quevenne and Gaultier de Claubry. A summary of the paper by the latter author is published in the *Bull. d. Acad. d. Med.*, 7 (1842). Quevenne regards the adulteration as unlikely, and thinks that it could not serve as a means of concealing added water but might be made to conceal skimming.

These French experimenters found that milk that had been adulterated by them with brain-matter presented properties different from those described by the anonymous writer in the *Gazette*.

A. H. Hassell, in his work on "Food Adulteration" (1876, p. 418) states that Mr. Queckett, the famous microscopist, exhibited drawings made from examinations of milk containing nerve tissue. It is further on record that Queckett stated before a committee of the House of Commons that he had found such structures in the sediment from a sample of milk examined about 1850, but had not observed it since. He submitted drawings of fields obtained from cows' milk with and without addition of calves' brains. As Queckett was an accomplished microscopist

and a scientific investigator, it does not seem likely that he would be mistaken on this point and he was evidently sure enough of it to make drawings of what he saw, and bring them to professional and official notice. We may, therefore, accept this record as one of trustworthy character. Hassell figures, on page 426 of the work above noted, a field showing the appearance of milk containing brain-matter, but does not state whether it is a copy of Queckett's drawings or from a sample prepared for the purpose.

Additional evidence as to this form of adulteration is presented in a communication by Sidney Gibbons, F. C. S., in the *Chemical News*, 33, 134 (1874). After a brief reference to the statements of Hassell and Queckett, Mr. Gibbons details an interesting experience of which the following is an abstract:

At a suburban hotel, near Melbourne, Victoria, frequent complaints had been made of the quality of the milk supplied, and on a certain date a sample was furnished to the chemist. As received, the sample had formed into three layers, the highest and lowest being thick and dirty gray, the middle one thin and colorless. When shaken, the mass became uniform and pulpy without frothing but stratified again on standing. Its specific gravity was 1022. The top layer did not closely resemble cream and contained but few fat-cells. The lowest stratum was darker, including dirt, sand, and vegetable matter. Much granular and clotted matter was present which was distinguished from fat by staining with magenta. There was no material amount of starch, though a few granules were present. Many peculiar cell-structures resembling brain-cells were seen under the microscope. Mr. Gibbons mixed some sheep's brain with milk and obtained optical appearances similar to those in the sample. He did not succeed, however, in making so smooth a mixture, possibly because the milkman had manipulated the material more elaborately. The fields observed agreed closely with the drawings given by Hassell.

From these reports it seems justifiable to record brain-matter, especially calves' and sheep's brains, as adulterations of milk that have occurred and may again occur, and therefore not to be overlooked in the analytic manuals.

Quevenne and Gibbons incline to the view that this form of adulteration is not dangerous to the public health, although, of course, not in any sense permissible. Such views may be easily excused when we consider how little was known, even at the date of Gibbons' report, concerning the diseases likely to be conveyed by milk. The view of the French and English chemists is based on the fact that brain-matter is a regular article of food but this loses its value when we remember that for such purpose the brains are cooked, which would be rarely the case when used for milk adulteration. Apart from the liability of the brain to contain virulent microbes, the localization there of certain stages of dangerous entozoa is by no means infrequent.

It is a question whether some of the special milk-laws would cover this form of adulteration but it is clearly prohibited by the general food-law in operation in most of the states of the United States, and butter made from such milk would come under the provisions of the national and state laws concerning oleomargarin.

It seems to me that this form of adulteration might easily escape notice. Brain-matter may give a rich creamy color to skimmed milk. All those who have written about the subject have spoken of the use of the microscope for detecting the adulterations, but in the routine inspection of milk-supplies, microscopic examination is rarely employed, and hence the adulteration might be overlooked. I have prepared samples by adding to skimmed milk, some calves' brain well pulped and strained through a close-mesh sieve. The nerve tissue can easily be seen with a moderate magnifying power. Evaporation in a milk-dish does not show anything different from ordinary milk, but the odor emitted when the residue is charred, preparatory to taking the ash, is suggestive of foreign animal matter. When the sample was treated with the mixture of pentyl-alcohol and hydrochloric acid, and then with strong sulphuric acid in the usual manner for rapid fat-determination, no peculiar reactions were noted. The proteids dissolved quite as readily in the acid as that from pure milk. The brain-pulp was found to be rapidly soluble without striking color in cold strong sulphuric acid.

HENRY LEFFMANN.

A New Form of Receiver for Fractional Distillation in Vacuo.[1]— While there are several forms of receivers which give satisfaction, when small quantities of liquids are fractionated *in vacuo*, most of them are open to the serious objection that it is necessary to destroy the vacuum to change receivers or to remove all or part of the distillate; these difficulties are entirely obviated by the apparatus shown in the figure.

The receiver consists of three separatory funnels sealed together, each of the three bulbs being provided with a side stop-cock, the uppermost having an additional one, which is connected with the vacuum pump.

The condenser tube enters into the top bulb, connection being made by a ground glass joint, for which a rubber stopper may be substituted if desired.

The *modus operandi* is obvious and almost automatic; the stop-cocks *a*, *d*, *e*, and *f* are closed, *b*, *c*, and *g* opened, the last being connected with the vacuum pump; the first fraction is collected in bulb A, the stop-cock *b* closed, and the second fraction collected in bulb B; by closing stop-cock *c*, the third fraction may be collected in bulb C.

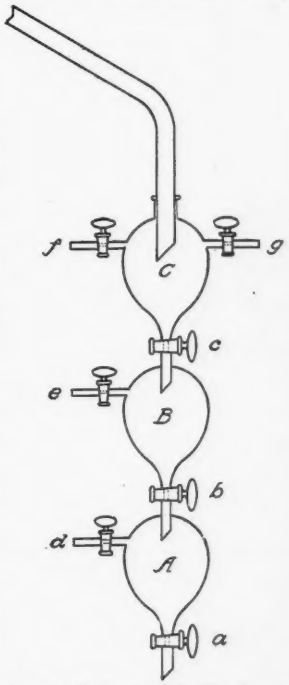
If it is desirable to separate the distillate into more than three fractions, or if the quantity of the distillate exceeds the capacity of the bulbs (which may be 1 liter each), the apparatus shows its special advantages.

Suppose the first fraction has been collected in bulb A and the second is being received in B; by opening stop-cock *d*, air is admitted into A, and the contents withdrawn through *a*; closing this and connecting *d* with a second pump, the vacuum is re-established in A. This point may be ascertained without the aid of a separate manometer by slightly opening *b* when, if the vacuum is less in A than in B, air will bubble through the liquid in the latter, while if the pressure in both bulbs is the same, or if it is less in A, the distillate will flow down to A, where it may be collected and drawn off as before, and the operation repeated *ad infinitum*.

With a little experience a receiver of a total capacity of three liters may be so manipulated as to distil several liters of liquid without interruption. In actual practice 40 liters are

[1] Read at the meeting of the Cincinnati Section, February 15, 1900.

regularly distilled in this way, and each fraction is made sharp and exact, as there is no disturbance of the vacuum.



With bulbs of smaller capacity, *e. g.*, 250 cc. each, it is unnecessary to employ more than one pump, since the vacuum will not appreciably diminish in the short time necessary to close *g*, disconnect the pump, attach it to *d*, and reestablish the vacuum in this bulb. A makeshift apparatus, embodying the principles of the one shown in the sketch, can be made in any well-equipped laboratory by connecting three separatory funnels by means of two-holed rubber stoppers, the other holes being occupied by bent glass tubes provided with stop-cocks or even rubber tubing and pinch-cocks.

For continued use the apparatus with the bulbs sealed together is to be recommended; the one described has been used almost daily by the writer and was made according to his designs by E. Greiner, No. 62 Centre Street, New York.

LUCIEN FOGETTI.

OBITUARY.

Hon. Nathaniel Peter Hill, of Denver, Colorado, scientific metallurgist, sometime professor of chemistry in Brown University, and later United States Senator from Colorado, was born in Montgomery, N. Y., February 18, 1832; he died in Denver, Colorado, May 22, 1900.

His father and mother were notable people in Orange County, N. Y. The father, a well-to-do and substantial farmer, had been lieutenant of cavalry in the war of 1812 and subsequently he held many offices of trust in his county where he was

respected as a man of superior abilities and high character.

When Senator Hill entered Brown University in the year 1853 he was a bright and promising young man, matured and developed by the responsibility which had come upon him, while yet little more than a boy, of managing the farm after his father's death. His interest in scientific agriculture led him to devote his chief attention to chemistry, and his progress in the subject was so rapid and so substantial that he was soon made an instructor, and in 1859, he was appointed professor of chemistry. He soon set about the securing of funds for a new chemical laboratory for the university, and he accomplished the work of its construction in 1862.

A little later his attention was directed to the freshly opened gold-bearing districts in Colorado. The field appeared so promising that in 1864 he resigned his professor's chair and turned his attention to practical metallurgy. He was early impressed with the necessity of *smelting* rather than *milling* the Colorado ores of Gilpin County, and he proceeded to make a careful study of the whole subject at the metallurgical works of Freiberg, in Saxony, and Swansea, in Wales. As a result, he formed a business connection with the noted house of Vivian & Sons in Swansea. In 1867 he organized the Boston and Colorado Smelting Co., a concern which has operated successfully for over 30 years and of which up to the time of his death he was the general manager. Professor Hill is acknowledged as the chief agent in the creation of that great industry which was necessary to the development of the mineral resources of his adopted State.

Space does not permit a full statement here of his achievements, but a partial enumeration of his official and business positions speaks volumes. He was mayor of Black Hawk, Colorado, in 1871; member of the Colorado Territorial Council, 1872–3; United States Senator from Colorado, 1879–1885; member of the International Monetary Commission, 1891; president of the United Oil Company, working in the Florence district; president of the Colorado Smelting and Mining Company, operating on an extensive scale in Montana; president of the Denargo Land

Company, engaged in developing real estate in the vicinity of Denver; principal owner of the *Denver Republican*.

As a chemist and scientific man Professor Hill was characterized by clear insight, great grasp of his subject, and sound judgment. His scientific work related chiefly to applied chemistry. During his professorship in Brown University he continually acted as expert and adviser in connection with chemical manufacturing establishments in and near Providence. But his life-work was in metallurgy, and the complex operations of his refining establishments near Denver are a monument to his power in the scientific treatment of metallic ores. As a university professor he was highly valued; he was not only recognized as a master of his subject and as a clear thinker, but more, he was loved by his pupils as a true gentleman. He was a remarkably able business man, having far-sightedness, wisdom, and courage. As a legislator he secured—as he merited it—a reputation for ability, dignity, and integrity.

Professor Hill married Miss Alice Hale, of Providence, R. I., a member of a remarkably able New England family, and a woman of superior powers and great sweetness of character. She survives him with three children: Crawford Hill (A. B., Brown University, 1885), Miss Isabel Hill, and Mrs. Gertrude (Hill) Berger.

J. H. APPLETON.

NEW BOOKS.

ANALYSE ÉLECTROCHIMIQUE. PAR EDGAR F. SMITH, Professeur de Chimie a l'Université de Pennsylvanie. Traduction par Joseph Rosset, Ingénieur civil des Mines. Paris: Gauthier-Villars. 1900.

The hearty favor with which this excellent little work was received on its first appearance by English-speaking chemists, and its translation into the two languages that can boast of more voluminous electrochemical literatures than the English, are doubtless sources of much gratification to its author. This French version, like the German that preceded it, is a clear and faithful translation of the second American edition of 1894. It contains a few changes and additions such as the separation of some of the platinum metals, analytical results, and some useful tables, yet these are hardly of sufficient importance to justify the

translator's claim "c'est donc en quelque sorte une édition originale paraissant en français que nous présentons." As a feature quite unusual in French books of this kind, we note that careful attention has been given to the spelling of the names of foreign authors. H. F. KELLER.

ERRATUM.

On page 139, line 10, of February, 1900, issue, for the formula "$[(CH_3)_3NH]_3TeCl_6$" substitute "$[(CH_3)_3NH]_2TeCl_6$."